LECTURES

IN

MEDICAL BIOCHEMISTRY

BY

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FOR

MEDICAL STUDENTS - POST GRADUATES AND

DENTAL STUDENTS

PART I

2006 - 2007

DEDICATED TO :

* THE SOUL OF MY PARENTS .
* ALL MY FAMILY MEMBERS FOR THEIR PATIENCE AND ENCOURAGEMENT UNTILL THIS BOOK WAS BORN .



PREFACE

* THIS BOOK IS SPECIALLY WRITTEN TO MEET THE NEEDS OF MEDICAL STUDENTS,POSTGRADUATES, AND DENTAL STUDENTS.
* BIOCHEMISTRY IS NOT A DIFFICULT SCIENCE,BUT NEEDS SIMPLIFICATION AND UNDERSTANDING WHOSE WERE TRIED IN THIS BOOK
* MANY UPDATING KNOWLEDGES IN MEDICAL BIOCHEMISTRYAND ITS RELATION TO BODY BIOCHEMISTRY OR HUMAN DISEASES WERE TRIED.
* FINALLY,THANKS TO ALL MEMBERS OF THE MEDICAL BIOCHEMISTRY DEPT.,FACULTY OF MEDICINE,ALAZHAR UNIVERCITY WHO SINCERELY SUPPORTED THIS BOOK.

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CONTENTS

CARBOHYDRATES

Definition:

Are polyhydroxyl organic compounds containing active aldehyde group (-CHO) as glucose, or active kitone (C = 0) group as in fructose.

Are derived from CO_2 & H_2O by photosynthesis.

Organic structure:

- Carbohydrates are compounds composed of C, H, and O; the latter two are present in the same ratio of water (H_2O = 2:1).
- $C_6 H_{12}O_6 = C_nH_{2n} O_n = C_6 (H_2O)6$

Importancc:

1. The main energy source donor for the body (60% of whole energy).,
2. Form about 50-60% of human diet (is cheaper source).
3. Enter in formation of nucleic acid in the cell (Ribose →RNA and Deoxyribose → DNA).
4. Enter in formation of connective tissues, tendons, skin and cartilage (mucopolysaccharides).
5. Enter in formation of some vitamins as riboflavin (B_2) contains ribitol, also vit. C is derived from acid sugar (gluconic acid).
6. Excess CHO is stored in liver as glycogen and in subcutaneous tissue as fat

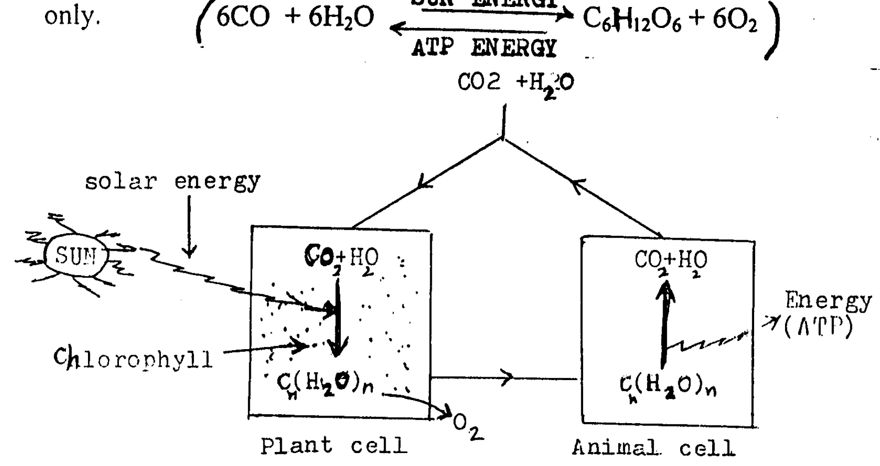
Carbohydrates as a source of energy:

Green plants and algae are able to use the energy in sunlight to synthesize carbohydrates from water and CO_2

during the photosynthesis.

Then these plants containing carbohydrates are eaten by human, ingested (glucose) and oxidized inside the cell yeilding H_2O, CO_2, and release of energy for body activity.

So, in this way the energy of sunlight can be utilized by animal kingdom through oxidation of green plants in the body providing energy inside the cell for its activities but external sunlight heats surface body only. $\left(6CO + 6H_2O \underset{\text{ATP ENERGY}}{\overset{\text{SUN ENERGY}}{\rightleftharpoons}} C_6H_{12}O_6 + 6O_2\right)$

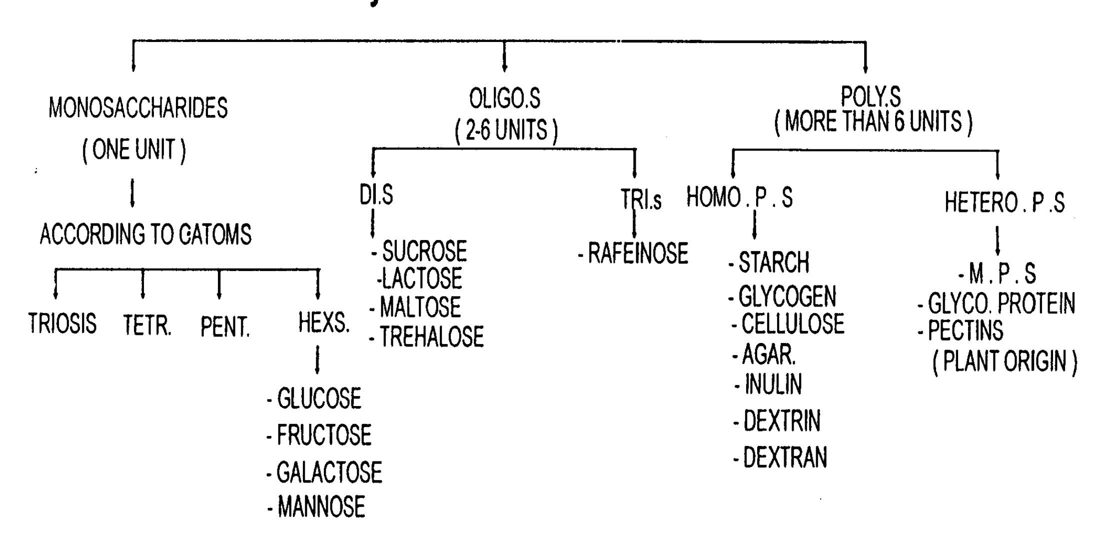


CHO(ENERGY) CYCLE.

*CHO is formed from CO_2 + H_2 (of H_2O).

*Free O_2 is liberated from oxygen of (H_2O).

Carbohydrates can be classifed into



I. Monosaccharides: (one sugar unit)

Every type of monosaccharides has aldo. form (-CHO) or Keto. form (-C = 0).

Pentosis: (ribose, deoxyribose, xylose)...Aldo.form.

(ribulose = Kito. form).

- Present in nucli of animal and plant cells.
- Enter in structure of DNA, RNA, and vit. B_2.
- When ingested not oxidized in body and excreted in urine.
- Don't give rise to energy, not fermentable by yeast.
- Pentosis can be formed in the body from glucose 6.p (Hexose monophosphate shunt) to enter in nucleic acid synthesis.

Hexosis: (glucose, galactose, mannose and fructose)

Classified according to free active group into:

1. Aldosis: has free aldhyde group (-CHO) as glucose (dextrose), galactose, and mannose.
2. Ketosis: has free ketone group (-C = 0) as fructose (laevulose).

Structure:

i. Simple straight formula:

D- Glucose	D.MANNOSE	D.GALACTOSE	D.FRUCTOSE
CHO	CHO	CHO	CH_2OH
H - C - OH	HO-C-H	H-C-OH	C=O
HO - C - H	HO-C-H	HO-C-H	HO—C—H
H - C - OH	H-C-OH	HO-C-H	H—C—OH
H - C - OH	H-C-OH	H-C-OH	H—C—OH
CH_2OH	CH_2OH	CH_2OH	CH_2OH

L-Glycerose (L-glyceraldehyde) — D Glycerose (D-glyceraldehyde)

- D. sugars:

  In which OH group above the last one is to the right; and orginate from D. glycerides sugar (hexosis parent).

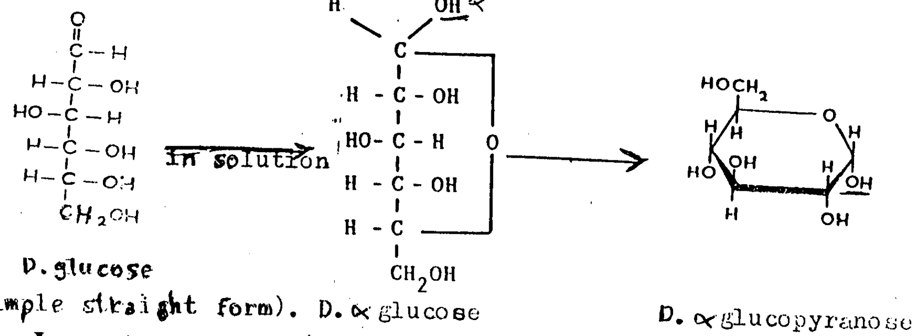
- L. sugars:

  In which OH group above the last is to the left side.

ii. Cyclic form formula:

- (OH) group of C_1, if to right $\rightarrow$ α. sugar.
- (OH) group of C_1, if to left $\rightarrow$ β. sugar.
- The 1-5 ring is called pyranose form as in glucose.
- The 2-5 ring is called furanose form as in fructose.

α D-Fructofuranose



D. glucose (simple straight form). D. α glucose (1-5 cyclic form) D. α glucopyranose form.

Importance:

1. Glucose:

* Is the blood sugar, oxidized in tissues to give energy.
* Is called grape sugar, in medicine called dextrose (dextrorotatory).

- Glucose is obtained from fruit juices, hydrolysis of: starch, maltose, and cane suger.
- Excess glucose, is stored in body as liver glycogen as store of glucose in body. And in subcutaneous tissyse as fat by lipogeneses.
- It is increased in blood in case of diabetus mellitus.

2. Fructose:

- Present in fruits, honey, fetal blood, and semen (its energy supply).
- In body is changed into glucose in liver and intestine.
- It is sweeter [(170 degree) than sacrose (100), glucose (75), galactose (16)] is called laevulose (laevorotatory).
- Commercial fructose is obtained from glucose with action of xylose isomerase enzyme (bacillus bacteria).

Xylose isomerase

Glucose ----------------------→ Fructose.

D.GLUCOSE		Fructose
CHO		CH_2OH
H-C-OH		C = O
HO-C-H		HO- C - H
H-C-OH	⟶	H - C -OH
H-C-OH		H - C -OH
CH_2OH		CH_2OH

3. Galactose:

- Produced by hydrolysis of lactose (milk sugar), and synthestized in body from glucose in mammary gland.
- Galactose diet, is changed into glucose in liver.
- Enter in structure of glycoproteins and glycolipids.

4. Mannose:

Obtained by hydrolysis of gum, enter in structure of glycoproteins.

General properties of monosaccharides:

I. Physical properties:

Physical properties of CHO depends upon presence of at least one asymetric carbon atom; (attached to four different groups; presence of double bond or similar groups interferes with it).

This gives rise to two phenomena:

A) Optical isomerism:

- Are isomers of sugar (D or L sugars), denotes the origin of the sugar; D. sugar originates from D. glyceraldhyde (parent sugar) in which (OH) group above the last group is to the right.
- L. sugar, in which (OH) above the last group is to the left.
- Number of isomerism is determined number of asymmetric carbon atom by (2^n), where (n) is number of asymmetric carbon atoms. Therefore, glucose has 4 asymetric carbon atoms (2^4) = 16 isomers. And fructose has 3 asymetric carbon atoms (2^3) = 8 isomers.

```
     CHO                    CHO
      |                      |
  HO- C - H              H - C - OH
      |                      |
     CH2OH                  CH2OH

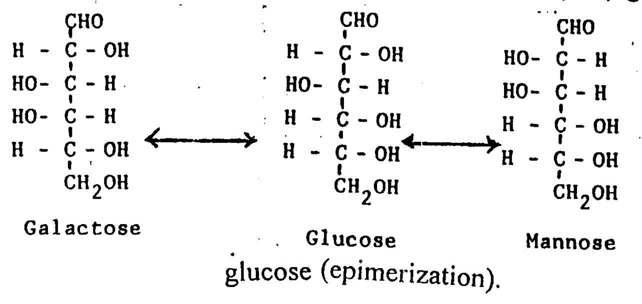
L.Glyceraldhyde        D.glyceraldhyde
```

B) Optical activity:

1. α and β anomers:

Is type of isomerism of monosaccharides, when it is present in cyclic form in solution.

The isomerism occurs in the carbonyl or anomeric carbon atom (C_1), yeilding two forms of sugar (α and β), in which (OH) group if to



glucose (epimerization).

α, β
CHO ← Anomer
H-C-OH ⎤
H-C-OH ⎦ epimer
HO-C-H
H-C-OH ← isomer (D&L)
CH_2OH
D-Gulose

right → α. sugar, if to left → β. sugar, this leads to phenomena of mutarotation.

2. Mutarotation:

Is change in degree of angle of polarized light by fresh solution of glucose (contains amounts of α and β glucose).

α. glucose has angle of rotation +112, but β. glucose has angle of rotation (+19), so if that solution is left; the α. glucose angle (+112) will decrease, while the β. glucose (+19) will increase to reaches the degree of equilibrium between α and β types (+52), where the solution because fixed at this degree.

3. Epimers:

Are isomers formed by interchange of the (-OH) group and (-H) on carbon atoms 2, 3, and 4 of glucose leading to formation of other sugars (epimers), as mannose and galactose.

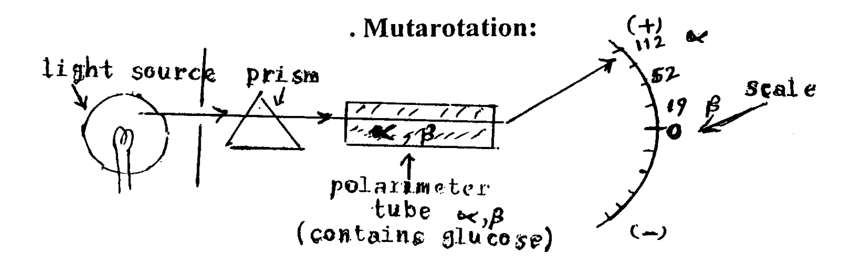
4. Dextro and laevorotatory:

Sugars are optically active (has asymmetric carbon atom), and cause deviation of the polarized light to the right (dextrorotatory; +) as glucose or to the left [laevorotatory; (-)] as fructorse, so (+) and (-) denote the rotation of the sugar. So, glucose(dextrose) is +52, while fructose(laevulose) is -92.

5. Racemic mixture:

When equal amounts of dextro and laevorotatory are present free in solution, the mixture has no optical activity (cancel each other), and called recemic or DL. mixture (inactive sugar); don't affects rotation.

. Mutarotation:

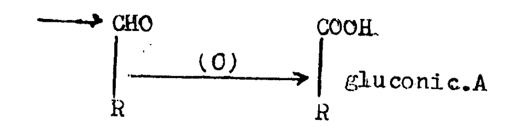


II. Chemical properties:

General:

- All carbohydrates are converted to glucose in body.
- All are fermented by yeast except galactose.
- All give positive molisch's test.

1. Oxidation:



a) simple oxidation:

Sugars are simply oxidized by bromine water into gluconic acid, which used in medicine as calcium gluconate injection.

b) Moderate oxidation:

Sugars are moderately oxidized by nitric acid ('HNO_3) into glucouronic acid, which acts as detoxicating agent in the liver, enter in structure of mucopolysaccharides.

R | CH_2OH $\xrightarrow{HNO_3}$ R | COOH glucouronic.A

c) Strong oxidation:

Sugars are strongly oxidized by H_2SO_4, into cyclic furfural compound, which is base of sugars identification(molish's test).

2. Reduction:

Sugars are reduced by reducing agent as Na. amalgam into the corresponding alcohol by adding 2H on its active group.

Glucose $\xrightarrow[NADP_2H]{+2H}$ sorbitol (its oxidation $\xrightarrow[NAD]{-2H}$ fructose in semen).

CHO | R $\xrightarrow{2H}$ CH_2 OH | R

Mannose $\xrightarrow{+2H}$ mannitol (used in brain odema).

Fructose $\xrightarrow{+2H}$ sorbitol and mannitol.

Ribose $\xrightarrow{+2H}$ ribitol (enter in riboflavin formation, vit. B_2).

(Sorbitol; is member of vit. B complex, and lipotropic factor).

Is nutritive sweetener, and act as osmotic laxative.

Its accumulation in eye lens of diabetic patients leads to cataract •

3. Ester formation:

Sugars react with phosphoric acid (H_3PO_4) to form ester (glucose 6p), which is the active sugar entering in different biochemical reactions (absorption and utilization).

→ CHO | CH_2OH $\xrightarrow[+P_i]{-H}$ CHO | CH_2O—P

4. Reducing property of sugar:

Monosaccharides are strong reducing compounds; can reduce the oxide of metals such as cupric hydroxide into cuprous oxide, due to presence of active free aldhyde or ketone group. Is base of Benedict test to detect sugars in urine.

cupric.OH (blue) — cuprous oxide(red)

$Cu(OH)_2 + Cu(OH)_2 + R{-}CHO \xrightarrow[(O)]{\text{Mono.s}} Cu_2O + 2H_2O + R{-}COOH$

5. Effect of alkalies:

- Week alkalies: Ca $(OH)_2$ and Ba $(OH)_2$; lead to interconversion of glucose to mannose or galactose (epimerization).
- Strong alkalies lead to decomposition of sugars into small fragments called caramel (polymerization).

6. Fermentation: $\left(C_6H_2O_6 \xrightarrow[zymases]{yeast} 2C_2H_5OH + 2CO_2 \right)$

* Glucose, fructose and mannose are easily fermented into ethyl alcohol and CO_2 by Baker's yeast, (zymases).

Glucose $\xrightarrow{zymase}$ glucose.6.p $\xrightarrow{oxid.}$ 2 pyruvate (CH_3. CO .COOH) $\xrightarrow{-2CO_2}$ 2 acetaldhyde (CH_3CHO) $\xrightarrow[2H]{reduction}$ 2 ethyl alcohol $C_2H_5.OH$.

* lactose, galactose and pentosis not fermented by yeast

Derivatives of monosarcharides:

1. Deoxy sugars:

- In which (-OH) group on C_2 is replaced by (H) atom; as in deoxyribose.
- Enter in formation of DNA.

```
  CHO                 CHO
   |                   |
H - C - OH         H - C - H
   |       -OH         |
H - C - OH ------> H - C - OH
   |       +H          |
H - C - OH         H - C - OH
   |                   |
  CH2OH               CH2OH

  ribose           deoxyribose
```

2. Amino sugars:

- In which (-OH) group on C_2 is replaced by $-NH_2$ group.
- Enter in formation of mucopolysacchardies as glucosamine, and in sialic acid as mannosamine.
- Also in activity of antibiotics as erythromycins (dimethyl amino sugar).

```
          CHO
           |
 -->  H  - C - NH2
           |
      HO-  C - H
           |
      H  - C - OH
           |
      H  - C - OH
           |
          CH2OH

      Glucosamine
```

3. Sugar acids:

Formed by oxidation of monosacchardies:

- In C_1 of glucose $\rightarrow$ gluconic acid
- In C_6 of glucose $\rightarrow$ glucouromic acid.

- In C_1 and C_6 of glucose → gluccharic acid. → 1 CHO —oxid.→ COOH ; → 6 CH_2OH → COOH

4. Sugar alcohols:

Is reduction of monosaccharides, giving rise to corresponding alcohols.

Mannose $\xrightarrow{+2H}$ mannitol, glucose $\xrightarrow{+2H}$ sorbitol, ribose $\xrightarrow{+2H}$ ribitol.

5. Acids of aminosugars (Sialic acid):

Formed by condensation of amino sugars (mannosamine), and organic acid (pyruvic and acetic acids).

Sialic acid enter in formation of glycoproteins, has immunological role & may be used as tumour marker.

Pyruvic acid: *COOH – *C = O – *CH_3
+
Mannasoamine: H - C = O; H_2N- C - H; HO- C - H; H - C - OH; H - C - OH; CH_2OH

→

Neuraminic acid: *COOH – *C = O – *CH_2 – H - C - OH; H_2N- C - H; HO- C - H; H - C - OH; H - C - OH; CH_2OH

Acetic acid: $+CH_3COOH$, $-H_2O$ →

Sialic acid (N-acetyl neuraminc acid): *COOH – *C = O – *CH_2 – H - C - OH; CH_3- C(=O) - HN- C - H; HO- C - H; H - C - OH; H - C - OH; CH_2OH

6. Glycosides:

Are formed by combination of active group of monosacchardes (-CHO), and the (-OH) group of second compound which may be other monosaccharides or other alcohol as (methy alcohol or sterol).

Other example, is cardiac glucosides (used in heart failure) in which β- galactose is attached to OH group of sterol.

Digoxin is cardiac glucosides (used in heart failure) in which galactose is attached to OH group of sterol.

Digoxin is present in leafs&seeds of digitals plant.

β. GALACTOSE → STEROL

7. Fucose = 6-deoxygalactose = methyl pentose:

Enter in structure of glycoproteins, has immunological role & may be used as tumour marker.

L-Galactose		L- Fucose
CHO		CHO
H -C- OH		H - C -OH
HO -C- H		HO- C - H
HO -C- H	→	HO- C - H
HO -C- H		HO- C - H
CH_2OH		CH_3

Disaccharides:

Are formed by combination of two molecules of monosaccharides, liked together by glycosidic bond.

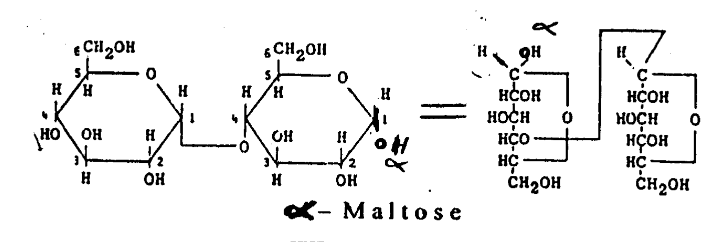
Disaccharides may be:i)reducing: has free aldehyde group as [CHO] as in maltose, lactose and cellobiose, so can be present in two forms α &β anomers .

ii) Non reducing:(has no aldehyde group) as sucrose and trehalose.

Types:

1. Maltose (malt sugar):

- Formed of two moles of D-glucose linked together by α 1-4 glycosidic bond .
- Is end product of starch digestion by amylase.
- Present in germinating barely.
- Is reducing sugar (has free aldhyde group), so present in α and β forms.



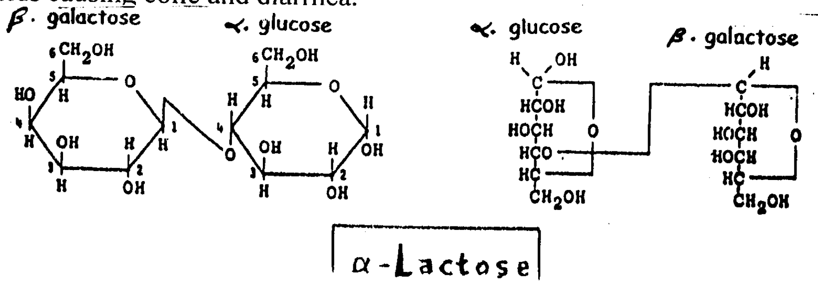
α- Maltose

2. Lactose (milk sugar);

- Is reducing sugar, not fermented by yeast.
- Present in urine during lactation.
- Formed from combination of α-glucose and β-galactose (1– 4 link)
- Present in two forms (α&β) in lactating female.
- Present in milk about (5 – 7 %).

* LACTOSE INTOLERENCE :

- Lactose is hydrolysed by lactase ---------→ glucose & galactose which is epimerized into glucose in small intestine, and easily absorbed.
- In lactase deficiency, lactose is moved to colon where is fermented by colonic bacteria (E coli) into CO_2 & H_2 and irritating organic acids causing colic and diarrhea.

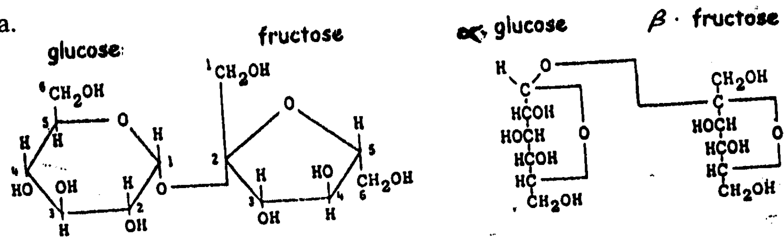


α-Lactose

• Now, Prehydrolysed lactose is prepared in milk powder.

3. Sucrose (cane or beet sugar):

- Is non reducing sugar (has no active group).
- Fermented by intestinal sucrase or yeast invertase into glucose and fructose.
- Sucrose if administrated intravenous is not utilized, it causes change in osmotic condition of blood, so used by clinicians in cerebral oedema.



Sucrose

4. Cellobiose:

Is disaccharides, reducing, obtained by hydrolysis of cellulose (β. glucose polymer); (1-4 link).

CELLOBIOSE

O-β-D-Glucopyranosyl-(1→4)-β-D-glucopyranose

5. Trehalose:

O-α-D-Glucopyranosyl-(1→1)-α-D-glucopyranoside

GLUCOSE C_1 + GLUCOSE C_1 -----> TREHALOSE

- Is non reducing sugar, attachment is α-glucose in C_1 to another α glucose in C_1(αC1 ⌐ 1)
- Present in fungi, and insect lymph.
- Yeast remains alive when dry in room temperature due to trehalose.
- So, new vaccines which contain trehalose as preservative can transported without need of refrigator.

Invert sugar:

Sucrose is dextrorotatory sugar (+), but after hydrolysis with invertase enzyme, it splits into equal amounts of fructose (-92) and glucose (+52) (invert sugar), which is laevorotary due to fructose content.

So, sucrose is inverted laevorotatory after hydrolysis with invertase enzyme.

$$\text{Sucrose } \underline{[\text{dextrorotatory}]} \xrightarrow[\text{enzyme}]{\text{invertase}} \text{fructose } (-92) + \text{glucose } (+52)\ \underline{[\text{lavevorotatony } (-)]}.$$

Invert sugar is formed from equal amounts of glucose and fructose, present naturally in bee honey.

Sweet degree:

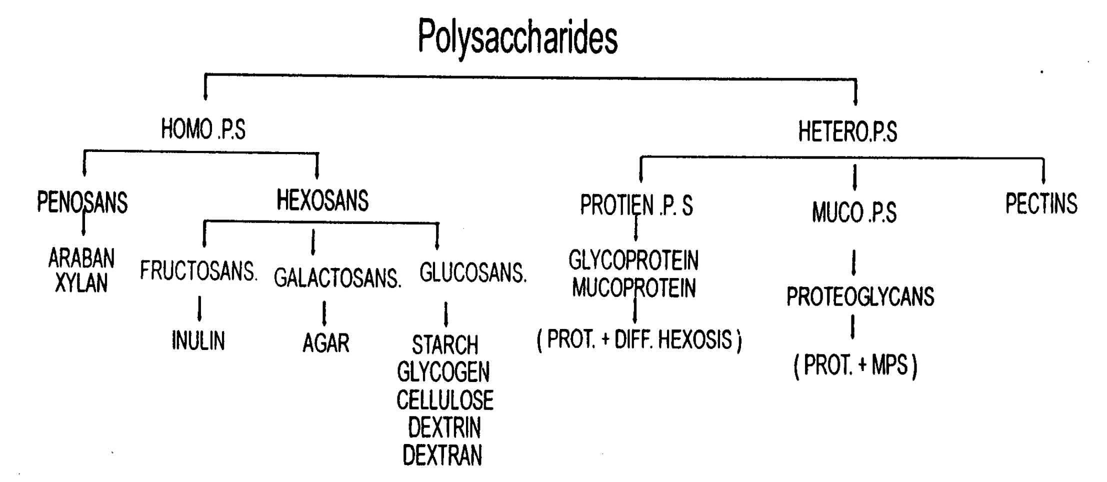
Sucrose (100), fructose (170), glucose (75), invert sugar (103), maltose (32), lactose (16), and galactose (16.).

Absorption rate:

Glucose (100), galactose (110), fructose (40), and pentose (20).

Polysaccharides

- Are high molecular weight sugars, formed of more than six units of monosaccharides.
- They are classified into homopolysaccharide (contain the same types of sugar units), and hetero or mixed polysaccharides (contain different types of sugar units).



I- Homopolysaccharides :

1- Fructosans (inulin) : *β fructose polymer.*

- Present in bulb of onion, artichokes, and chickory.
- Is combination of fructose units, β 1 – 2 link in straight chain
- Used in renal function test.

2. Galactosans (Agar-Agar):

- Is combination of galactose units, present in sea weeds.
- Used in culture media (in agar plates), prevent constipation by (imbibition) of water from colon.
- Not digested by humans.

3. Glucosans :

Is combination of α glucose units :

a) starch (α-glucose polymer):

- Sources: Its main source is plant origin: Cereals, potato, bananas,....
- Under microscope : Starch molecule is formed of two layers:
  i. Amylose; is the inner layer, form 20% of granule starch, is straight chain linked in α1-4 bond, soluble in cold water -------→ Clear solution
  ii. Amylopectin, is the outer layer, form 80%, of starch molecule, is branched chain, linked in α1- 4 link at straight chain and at branches in α 1- 6 link , is insoluble in cold water --→ Opalescent solution.
- **Hydrolysis:** By α.amylase enzyme gives rise to starch → amylodextrin → erythrodextine → achrodextin → maltose. (smallest size)
- Starch with acid hydrolysis -------→ glucose units
- α. amylase present in human & β amylase present in plants.
- starch --α amylase--→ maltose --maltase--→ glucose
- Gives blue colour with iodine.

80% Amylopectin

Amylose 20%

b). Glycogen (α-glucose polymer):

* **Source:** animal starch (10% of liver weight, and 1 – 2 % of muscles).
* Structure: Is high molecular weight, highly branched molecule; tree like.

- Link is α1 – 4 at straight chains, α 1 – 6 at branches.
- It gives red colour with iodine.

***Importence:** Is main store of carbohydrate in body, (can supply energy for about 18 hours)

c). Dextrin (α-glucose polymer):

- Is product of starch hydrolysis, branched.
- Has adhesive property (form sticky or gummy character)
- Starch hydrolysis (dextrin & maltose) used in infant feeding.

d). Dextrans (α-glucose polymer):

- Are produced by bacteria, branched; link is 1-3 at straight chain, 1-6 at branches, grow on sucrose culture media with gram +ve cocci .
- They polymerize glucose of sucrose ------→ dextrans

Used as plasma substitutes, is highly viscous and slowly degraded, persist in blood for many hours.

e). Cellulose (β-glucose polymer):

- Is straight chain, formed of β-glucose units, attached at 1-4 link.
- Is present in plants, framework of leafy vegetables and bran.

•• Cotton is purely cellulose, it contains from 300 – 1500 β glucose residues

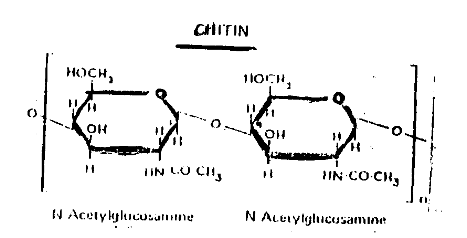
- It gives no colour with iodine.
- Is not digested in humans (has no cellulase enzyme), so it increases bulk of stool → stimulates movements → prevents constipation.
- Gives sense of fullness so may be used in obesity.

- Fibers present in skin of orange,peel of apples is more soluble cellulose fibers which decreases glucose and cholesterol absorption.

N.B :

* All homopolysaccharides are α . glucose, branched, except cellulose is β – glucose, and straight chain.
* Chitin :

  Is polymer of N. acetyl glucosamine residues, present in skeleton of insects & spiders, its link is 1-4 straight chain.



II- Mixed (hetero polysaccharides) :

Are combination of different types of sugar units, such as glycoprotein's (mucoproteins), glycosaminoglycans (mucopoly-saccharides) and pectins in plants.

1- Glycoproteins :

* Are different types of sugar units associated with proteins.
* Carbohydrate units are mostly hexosis (galactose, mannose) and amine sugar (N. acetylglucosamine) which acts as link between sugar units and protein, also fucose and sialic acid are present.
* The linkage between carbohydrates and protein may be :

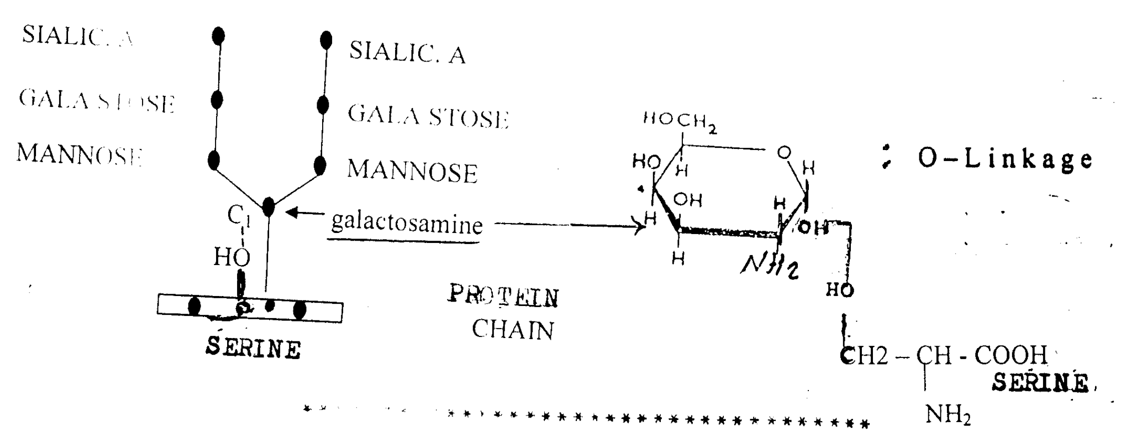
i) O. linkage : (C ⟶ O)

Attachement of C_1 of amine sugar moity with (OH) group of serine in protien chain.

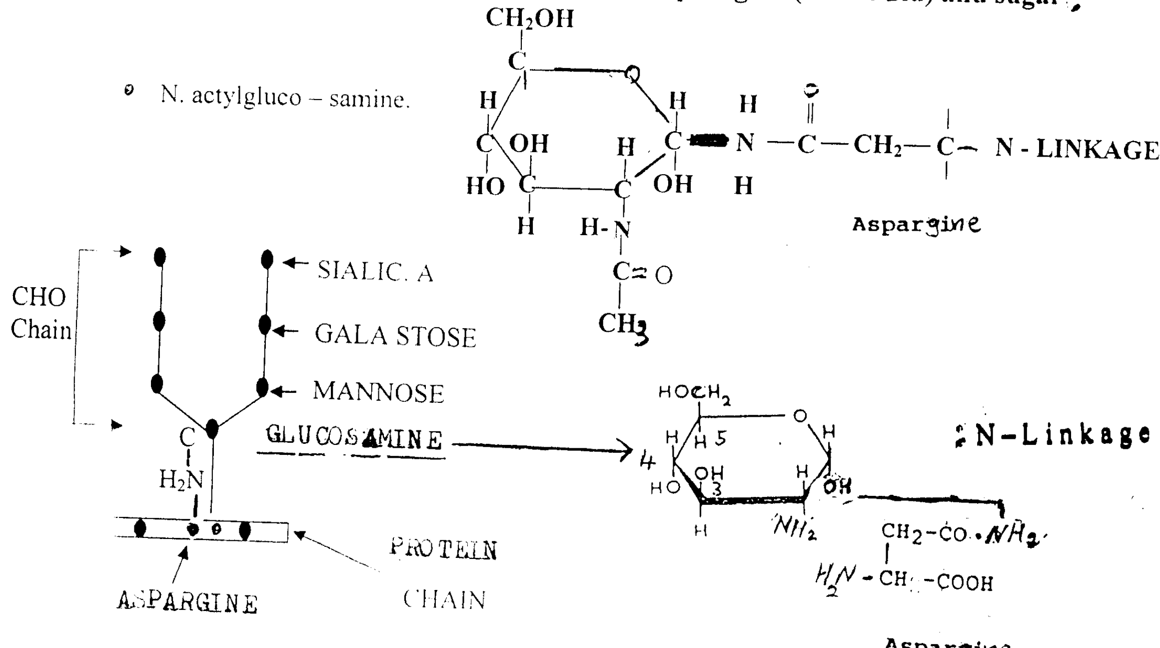
ii) N. linkage : (C ⟶ N)

* Attachement of C_1 of amine sugar moity with (NH_2) group of aspargine in protien chain.
* Glycoproteins are present in gamma globulins, some hormones, and cell receptors where they perform biological functions.
* Mucoproteins are type of glycoproteins, but has little protein and more hexosis, as haptoglobin, (binds to free Hb). Its carbohydrates content is more than 4%, but glycoprotiens is less than 4%.

1) O- linkage : between – OH at serine & sugar:



2) -- Linkage: between – N of amide group of asparagine (amino acid) and sugar :



2- Pectins :

* Are heteropolysaccharides (arabinose + galactose + galactouronic acid).
* Are present in fruits, and bulb of apple.

*Pectins are diet fibers,more soluble, form gel like substance.

*It decrease absorption of cholesterole

* Diet fibers(cellulose &pectins)decrease incedence of cancer colon.

3- Glycosaminoglycans (mucopolysaccharides) :

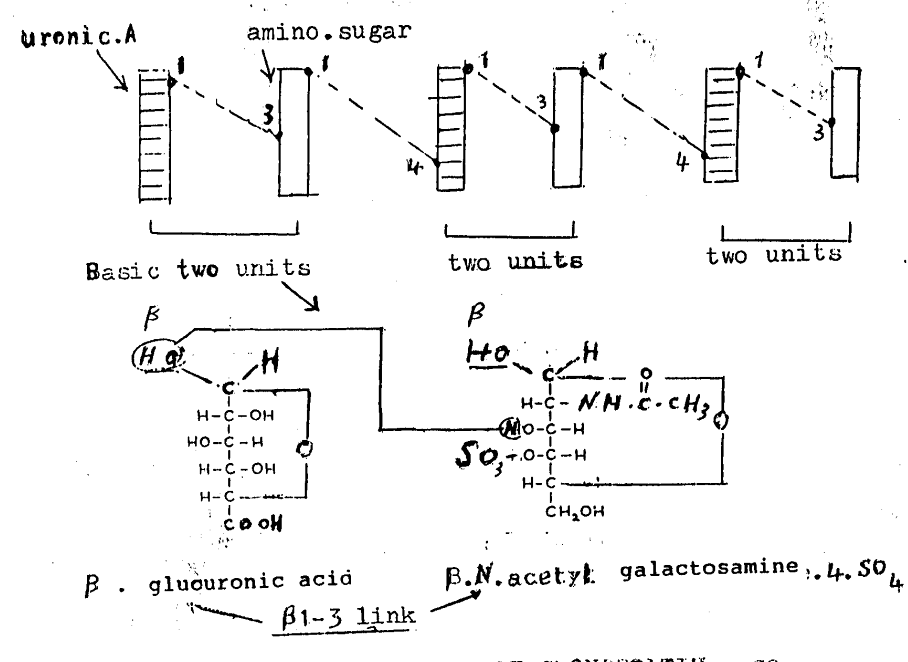
Are heteropolysaccharides which contain acid sugar and amine sugar, present in ground substance and connective tissues, skin, form straight chain.

Types	Site and function	Structure			
		Uronic acid	Amino sugar	Sulphatic as HSO4	Acetic acid CH_3COOH
1. Chondroitin 4-SO4 [repeated two units (β1-3 bond)]	Cartilage, bone, C-tissue has protective function	β-glucouronic	β-galactosamine	+	+
2. Muoitin-SO4	Mucous secretions of respiratory and digestive tracts (mucin)	β-glucouronic	β-glucoiamine	+	+
3. Hyal-uronic acid two mol (β1-3 bond) repeated (β1-4 bond) units	Synovial fluid, umbilical cord, vitrous humour, act as lubricant in joints, is highly viscus	β-glucouronic	β-glucsamine	-	+
4. Heparin two mol (α1-4) repeated (α1-4) units	Intracelluary in mast and basophill cells, small vessels of lung and liver [acts as anticoagulant]	α-glucouronic	α-glucosamine	+++	+
5. Dermatan. SO4 (α1-3 bond). (β1-4 bond)	Skin, tendons, heart valves, has weak anticoagulant action	α-id-glucouronic	β-galactosamine	+	+
6. Keratan. SO4 ((β1-4), (β1-4)	•Cornea, skeletal tissues •Play role in cornea transparency	β-galactose	β-glucosamine	+	+

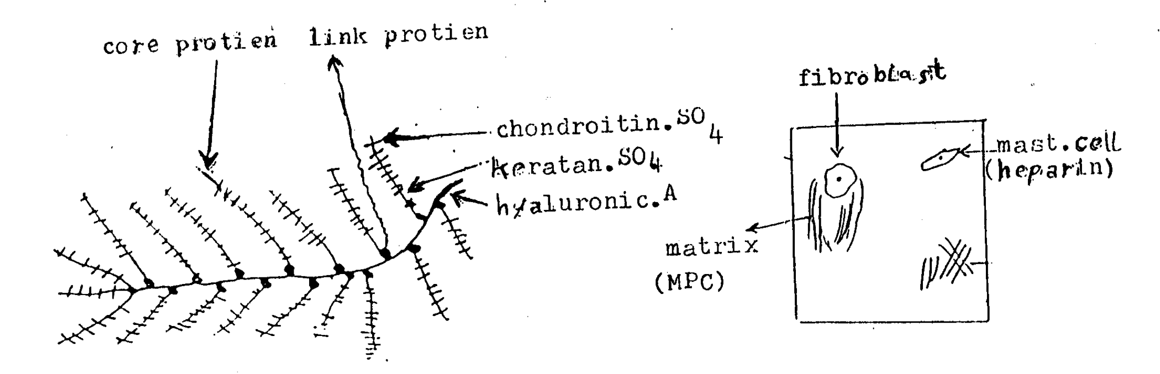
* Structure of glycosaminoglycans :

* The basic unit of glycosaminoglycans is formed of two repeated units of sugar acid (glucouronic acid) and (amine sugar) forming straight chain.
* Glucouronic acid is present in all types of glycosaminoglycans except dermatan- So4 contains its epimer id-glucouronic acid, in which(C_5 , C_6 are reversed).
* Glucosamine is present in all types except dermatan – So_4 and chondriotin – So_4 whose contain galactosamine.
* Amine sugar is usually acetylated (CH_3 COOH) at amine group (C_2) in all types of glycosaminoglycans.
* Sulphate group (Ionizid H So_4^- + H^+) is present in all types except hyaluronic acid (non sulphated). Sulphate group is located at C_4or C_6 of amine sugar.
* The basic unit of glycosaminoglycans is linked mostly by β 1-3 bond, except heparin & keratin. So_4 (1 – 4 link).
* The basic unit are repeated in long chain by 1-4 link.
* All glycosaminoglycans has no hexosis, except keratin – So_4 has galactose units.
* Glycosaminoglycans has ability to bind large amounts of water forming gel like substance.
* Glycsaminoglycans are negatively charged due to presence of carboxyl group (COO^-) and sulphate group So_3^- leading to stretched conformation of glycosaminoglycan chain , high viscosity of surrounding tissues and slippery consistency in mucous and synovial fluid.

STRUCTURE OF GLYCOSAMINOGLYCANS



BASIC TWO UNITS OF CHONDROITIN.4.SO4



(PROTEOGLYCAN STRUCTURE IN BOVINE CARTILAGE).

(SECTION OF CONNECTIVE TISSUSE).

* All glycosaminoglycans are located extracellulary, except heparin is intracellulary in mast cells.
* Proteoglycans : are glycosaminoglycans associated with proteins

Biochemical function :

* The main function is supporting and protection of connective tissues, bone, and cartilage.

1- Heparin :

* acts mainly as anticoagulant (prevent thrombus formation).

*Heparin increases activity of lipoprptien lipase, so clear circulation from the turbid chylomicrons

2- Hyaluronic acid :

- Is the largest glycosaminoglycans, its mol wt is about 10^6, highly viscus, acts as lubricant in joint,
- Hyaluronic acid is hydrolysed by hyaluronidase enzyme (presemt in bacteria and some viruses) which destructs hyaluronic acid decreasing its viscosity and increase invasion power of bacteria and viruses, so this enzyme is called spreading factor.
- The enzyme is present also in sperms to facilitate its penetration to ova.
- Hyaluronic acid if increase, may permit tumour cells to migrate in extracellular matrix to facilitate spreading of tumour cells.
- Salicylates inhibit hyaluronidase enzyme.
- Hyaluronic acid may keep temperature in knee synovial fluid constant in spite of its moving

3- Dermatan So_4 :

* Binds to plasma lipoproteins LDL, and may play role in development of the atheroscloretic plaque.
* Skin wrinkle of face may develop with age due to changes of some glycosaminoglycans.

*Present in sclera of eye ,giving its overall shape.

4- Chondroitin – So_4 :

* Protects cartilage and bone, it decreased in cartilage with age, leading to osteoarthrosis.

5- Mucoitin – So_4 :

* Occures in mucus secretion of respiratory and digestive tracts for protection of endothelium.

6- Keratan – So_4 :

* Occures in cornea and skeletal muscles, may play role in corneal transparency.

***** Mucopolysaccharidosis :**

* Is inborn error disease due to lacking of degradation enzymes as :

1- Hyaluronidase→act upon ——→ hyaluronic acid.
2- Glucouronidase→remove→glucouronic acid.
3- Sulfatases ——→remove→sulphate group.

This lead to accumulation of mucopolysaccharides which is increased in urine, the patient suffers from bone deformity & deformed facial features.

Glycoproteins	Glycosaminoglycans (mucopolysaccharides)
* Cantain hexosis	* Has no hexosis
* Has amino sugar	* Has amino suger
* Has more protein (about 95%)	* Has little protein (about 5%).
* Contain no So^{-}_4 group	* Has So^{-}_4 group
* Has no uronic acid	* Has uronic acid (glucouronic.a)
* Present in gamma glob, some hormones, and cell receptors	* Present in supportive tissues and connective tissues
*Has short branched chain (2-10 units)of CHO.	*Has long non branched chain (more than 50 units)

Protein Chemistry

Structures & Functions Of Amino Acids
Peptides, Proteins, Myoglobin and Hemoglobin

I) Amino Acids:

Definition:

Are small organic biomoleculas (has biological function) containing at least one amino group (-NH2) and one carboxylic gourp (-CooH) attached to the α carbon atom, they form the basic unit of proteins.

α Amino acids differ from one another by the chemical composition of their R (groups side chain).

General Structures:

- All amino acids in human proteins are L. amino acids; (its amino group-NH2 is to the left side of the α asymmetric carbon atom).
- D. amino acids are present in cell wall of bacteria and antibiotics (its amino group is to the right).
- The number of L. α. amino acids in all human proteins is about 20-22 types only, in nature there is about 300 amino acids.
- The body can synthetize 10 types of amino acids only (unessential amino acids), so human diets should contain th remain 10 essential amino acids for proper growth and health.

$$H_2N-\overset{\displaystyle R}{\underset{\displaystyle H}{\overset{|}{\underset{|}{C}}}}^{\alpha}-COOH$$

L.ALPHA AMINO ACID

Biomedical Importance:

(1) Amino acids form the basic unit of peptides and proteins, which perform biological functions (hormones, enzymes, etc.)

(2) Some amino acids act as nerve transmission and regulators (qlutamate and GABA).

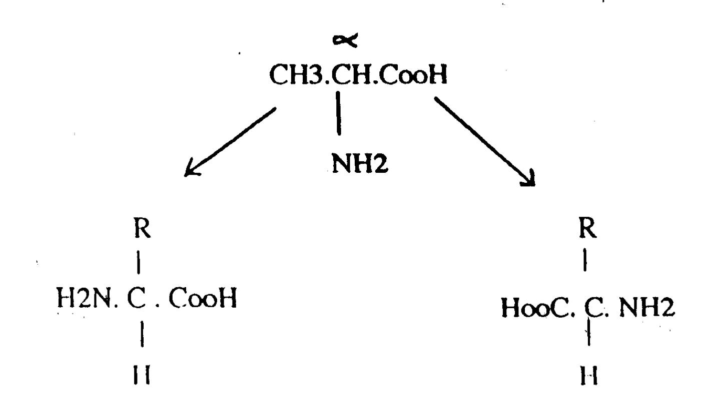
(3) Formation of some hormones (thyroxine, catecholeamines and melanin) from tyrosine.

(4) Formation of urea (to get rid of toxic ammonia) by orinithine and citrulline.

(5) Glycine gives rise to bile salts ,Hb,cr~p,glutathione, and acts as detoxicating agent in liver.

Properties of Amino Acids:

(1) All amino acids are optically active except glycine; has alpha asymmetric carbon atom attached to 4 different groups leading to formation of L & D amino acids.



L. α . alanine in humans

D. α . alanine in bacterial wall

(2) Relation Of pH to amino acid charges:

Amino acids has two ionizable groups; the carboxy (-CooH) and the amine (NH2) groups.

i) In physiological solution (pH 7 - 7.4)

One group is charged and the other is uncharged, and are present in proton balance.

R.[CooH] <-----------> R[Coo⁻] + H^+

R.[$NH3^+$] <-----------> R[NH2] + H^+

ii) In Low pH (acidic) Solutions:

The R < CooH / NH3+ form is predominant, and its charge is +ve, and run to cathode in an electric field.

iii) In High pH (alkaline) Solution:

The R < Coo^- / NH2 form is predominant, and its charge is -ve, and run to anode in an electric field.

Isoelectric Point (PI):

At certain pH of an amino acid, both carboxyl and amino groups are equally charged, so the net charge is zero, the amino acid cannot migrate in an electric field, and easily precipitated. R < Coo^- / $NH3^+$ or, defined as the pH at the midway between the PK values of the carboxy and amino groups. [$pI = \frac{PK1 + PK2}{2}$]

Amino acid molecule in this condition is attained the Zwitter ion (although charged, has no net charge).

$$^{+}H_3N - \underset{H}{\overset{R}{\underset{|}{\overset{|}{C}}}} - Coo^-$$

.... *pK:*

Is -- log of acid [H] dissociation constant of weak acid; or the pH at which the conc. of acid & its conjugate base is equall.

Classifications of the Amino Acids

(I) Amino acids may can be classified according to Polarity of R. Groups:

Amino acids may be classified according to polarity of their R groups into polar & non polar amino acids.

(1) Polar Amino Acids:

- In which the R. group attached to α carbon atom is highly charged.
- Polar amino acids has tendency to associate in polar solvents as water and ethanol, but insoluble in non polar solvents as benzene and ether.
- Polar amino acids are called hydrophilic amino acids.

Types:

Polar (hydrophilic) amino acids include:

Argenine, aspartic acid, aspargine, cystiene, glutamic acid, glutamine, glycine, histidine, lysine, serine and threonine.

(2) Non Polar Amino Acids:

- Their R. groups has little charge difference.
- Non polar amino acids has tendency to avoid water, but more soluble in non polar solvents.
- Non polar amino acids are called hydrophobic, and include:

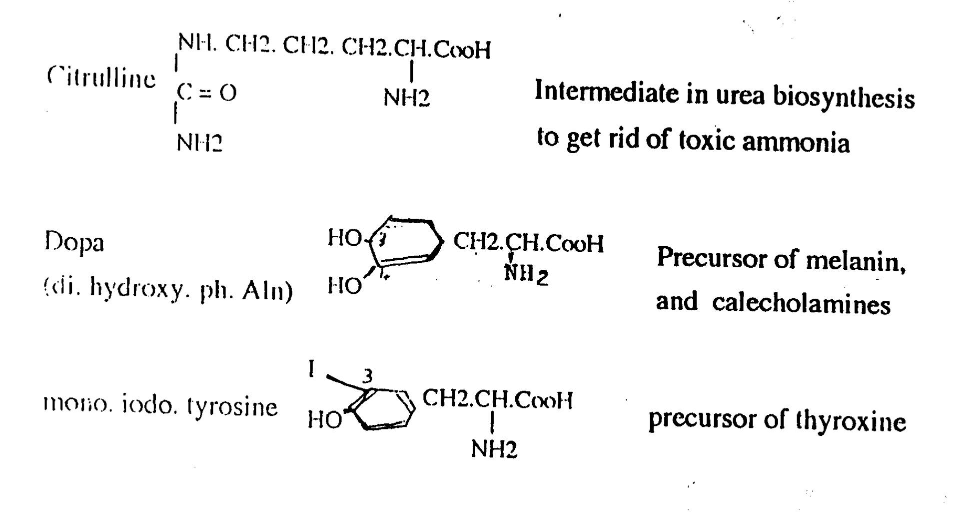
Alanine, leucine, isoleucine, methionine, ph.alanine, proline, tryptophane, tyrosine, and valine.

(II) Classification according to amino acids structure in human proteins :

Name	Formula	Comments
(1) Aliphatic side chain amino acids:		
Glycine	$H.CH(NH_2).COOH$	Simplest amino acid, optically inactive, enter in formation of Hb, glutathione, cr~p, and bile salts, present in gelatin (25%), possesces sugar taste.
Alanine	R: CH_3, α: CH — $CH_3.CH(NH_2).COOH$	Substrate for alanine transaminase(Gpt, Alt)
Valive	$(CH_3)(CH_3)>CH.CH(NH_2).COOH$	Essentool amino acid, branched R group.
Leucine	$(CH_3)(CH_3)>CH.CH_2.CH(NH_2).COOH$	Branched R group, hydrophobic, keto-genic.
Isoleucine	$(CH_3.CH_2)(CH_3)>CH.CH(NH_2).COOH$	Essential amino acid, branched R group.
(2) Amino acids with hyorxyl groups (OH) :		
Serine	$CH_2(OH).CH(NH_2).COOH$	Give rise to colamine base in phospho lipid cephalin, present in phosphopro-teinof milk casein .
Threonine	$CH_3.CH(OH).CH(NH_2).COOH$	Essential amino acid.
Tyrosine	$HO-C_6H_4-CH_2.CH(NH_2).COOH$	Intermediate in synthesis of, melanin, thyroxine and catecholamines.
(3) Amino acids containing sulfur atoms :		
Cystiene	$CH_2(SH).CH(NH_2).COOH$	Enter in formatron of glutathione, tau-rine of bile salts.
Methionine	$CH_2(SCH_3).CH_2.CH(NH_2).COOH$	Methyl donor, enter in formaton of cr~p
(4) Acidic amino acids (contain two carboxylic groups or their amides) :		
Aspartic acid	$HOOC.CH_2.CH(NH_2).COOH$	Sabstrate for GOT
Aspargine	$H_2N.C(=O).CH_2.CH(NH_2).COOH$	Participates in linkage of CHO to pro-tein in glyroproteins.
Glutamic acid	$HOOC.CH_2.CH_2.CH(NH_2).COOH$	Substrate for GOt &GPT, and gives rise to GABA.
Glutamine	$H_2N.C(=O).CH_2.CH_2.CH(NH_2).COOH$	Storage form of ammonia in body

Name	Formula	Comments
(5) Basis amino acids (Contain two amino groups) :		
Argenine	H . N . CH_2 . CH_2 . CH_2 . CH . COOH (with H_2N-C=NH on N; NH_2 on CH)	Involved in urea synthesies
Lysine	CH_2 . CH_2 . CH_2 . CH_2 . CH . COOH (with NH_2 on first CH_2 and on CH)	Essential amino acid
Histidine	[imidazole ring, HN, N] CH_2 . CH . COOH (NH_2 on CH)	essential , gives rise to histamine.
(6) Amino acids containing aromatic (benzene) ring:		
Phenyl alanine	[benzene ring] CH_2 . CH . COOH (NH_2 on CH)	Essential, gives rise to tyrosine
Tyrosine	HO-[benzene ring] CH_2 . CH . COOH (NH_2 on CH)	Intermediate in synthesis of thyroxine, catecholamines, and melanin .
Tryptophan	[indole ring, NH] CH_2 . CH . COOH (NH_2 on CH)	Precurssor of nicatinic a cid, gives rise to serstoniu , its indole ring causes fae-col odur.
(7) Imino acids (contain - NH instead of NH_2) :		
Proline	[pyrrolidine ring, NH] -COOH	Constituent of collagen and gelatin, has imino group (NH) .
Hydroxy proline	HO-[pyrrolidine ring, NH] -COOH	Present in collagen; its urine output is an index of bone matrix metabolism.

(III) Amino acids not included in proteins (has no role in protein formation) but has biological functions in humans:



(IV) Non Alpha Amino Acids With Biological Functions (enter in other biological compounds, not in protein formation):

	Structure	Function
β. alanine	β α CH2. CH2. CooH | NH2	Part of coenzyme A, and panto thenic acid vitamin.
Taurine	CH2. CH2. SO3H | NH2	Forms bile salt (taurocholic acid).
GABA	δ β α CH2. CH2. CH2. CooH | NH2	Regulates C. N. S. activity , acts asinhibitory neurotransmmitor in brain.

(II) Nutritional Classification:

Amino acids can be classified according to their requirements to body:

(1) Essential Amino Acids:

- Can't be easily synthetised in body, so should be supplied in the diet for optimal growth and health, e.g. valine, isoleucine, tryptophan, arginine (in growing children only) leucine, lysine, threonine, methionine, ph. aLn, and histidine.

(2) Non Essential Amino Acids:

- Can be easily synthetized in the body from CHO (pyruvate $\xrightarrow{\text{transamination}}$ alanine), or from essential amino acids [ph.aln $\xrightarrow{\text{hydroxylation}}$ tyrosine].
- The body will not suffers, if these amino acids are abscent from the diet.

(III) Metabolic Classification:

Amino acids can be classified according to their metabolic pathway in the body, some are converted to glucose [glycogenic amino acids] others are converted to ketone bodies; aceto acetyl CoA [Ketogenic amino acids], the rest is converted to both glucose & Ketone bodies [ketogenic & glycogenic amino acids].

Ketogenic & Glycogenic Amino Acids	Ketogenic [fat]	Glycogenic
L. Tyrosine, ph. Aln, Tryptophan, Lysine, isoleucine	L. Leucine	L. Alanine, arginine, aspartate, cystine, glutamate, glycine, histidine, proline, hydroxy proline, methionine, serine, threonine, and valine.

Formation Of Peptide Bond

- Two amino acids can be linked together by formation of peptide bond, through reaction of carboxyl group (-CooH) of one amino acid and the amine group (-NH2) of another one. One nolecule of water is removed.
- Carboxyl group (-CooH) should be activated first by ATP.
- [R.CH.CooH + ATP --------------> R.CH.CO - AMP] (-PPi written above the arrow; NH2 attached to the CH)

```
         R1              R2                                    peptide bond
         |               |                               R1  ┌────↓────┐ R2
         |               |                               |   | O       |  |
                                                         |   | ||      |  |
• H2N.C.CooH    +    H2N.C.CooH    -H2O      H2N     C ─┼─ C ── N ─┼─ C ─ CooH
         |               |        ---------->            |   |       |  |  |
         H               H                               H   |       H  |  H
                                                             └─────────┘
  [Amino Acid]     [Amino Acid]                              [Dipeptide]
```

Detection of Amino Acids:

(1) Ninhydrin Reaction:

- Oxidized, ninhydrin causes oxidation and decarboxylation of amino acids with libration of NH_3 which reacts with reduced ninhydrin giving blue colour complex, and an aldhyde.

R. CH. CooH + Ninhydrin [O] ------------> CO2 + R.CHO + NH3 (NH2 attached to the CH)

NH3 + reduced ninhydrin ------------> blue complex colour

- The colour can be measured at 570 nm wave length.
- Peptides also react but more slowly.
- Proline & hydroxy proline give a yellow colour (has imino group instead of amine group).

(2) Fluroscamine Reaction:

Is more sensitive than ninhydrin (detect in nanogram amounts), it forms a complex with amines (glutamine, histamine, ...) other than amino acids.

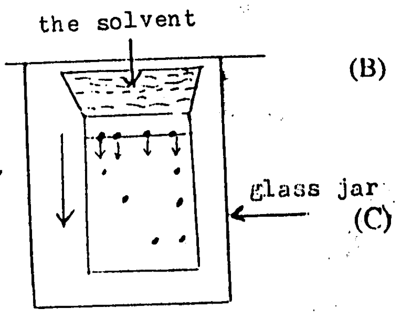
Separation Of Amino Acids

(I) Paper Chromatography: [according to polarity & M. wt]:

Is separation technique for a mixture of amino acids.

Procedure:

(A) Strip of cellulose filter paper separating media is marked with pencil about 5 cm from one end, and samples are applied at different points in addition to known standards.



(B) The strip is suspended in a jar containing a polar solvent as [butonol (12); acetic acid (3): water (5)], and left over night.

(C) The solvent containing amino acids migrates down (descending chromatography) and the amino acids are separated according to its polarity and molecular weight for different sites.

(D) The strip is dried and amino acids are detected by 0.5% ninhydrin in acetone, followed by heating 90-110 0C for 3 minutes, and amino acids are identified comparing of that standards.

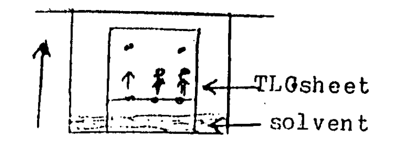
- Amino acids can be identified also by knowing the Rf values of different amino acids.

[Rf; is the ratio of distance travelled by amino acid to that by the solvent front, each amino acid has a specific Rf in a given solvent].

(2) Thin Layer Chromatography (TLC):

Is ascending procedure, the support media is silica gel or sephadex, they are spread on thin layer glass plates 20 X 20 cm, or on plastic sheets.

Advantages:



(1) Sharp good separation than paper chromatography.

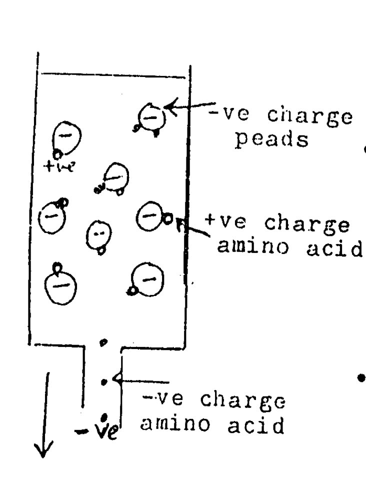
(2) Rapid separation time (3 hours), in paper chromatography (16 h.)

(3) Ion Exchange Chromatography: (according to net charge difference):

Principle:

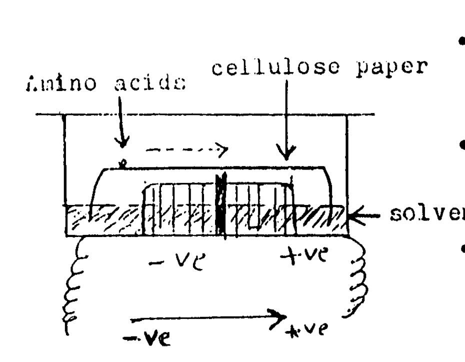
Amino acids mixture can be separated by ion exchange chromatography in which amino acids mixture is applied to a coloumn containing the -ve charge carboxy methyl cellulose peads (resin) for fractionation of positive charged amino acids (present in acid buffer).

Procedure:



- Amino acids mixture in acid media is +ve charged and bound to the -ve charged resin in the coloumn.
- By increasing the pH and ionic strength of the media (to be alkaline), the carboxyl groups of the amino acids lose its H^+ and thus turned to -ve charged amino acids and are released from the -ve charged resin. [CooH -----> Coo^- + H^+]
- Each amino acid emerge from the coloumn at a specific pH and ionic strength.

(4) High Voltage Electrophoresis: [according to charge & mol weight]:



- High volt is utilized 2000 - 5000 volt.
- Separating media is cellulose acetate paper, in which amino acids mixture is applied.
- In alkaline buffer the amino acids are -ve charged and migrate to anode.
- After complete run (0.5 - 2h) the strip is stained with ninhydrin and the amino acids are identified.

(5) Precipitation:

- Amino acids are easily precipitated at its isoelectric point [PI].
- Each amino acid has its own (PI), so mixture of amino acids can be easily separated by changing the pH of amino acid containing solution according to pI of each amino acid.

II) PEPTIDES

Definition:

A peptide consists of 2 or more amino acids (less than 10) linked by a peptide bond.

Polypeptide are long peptide chain (less than 100).

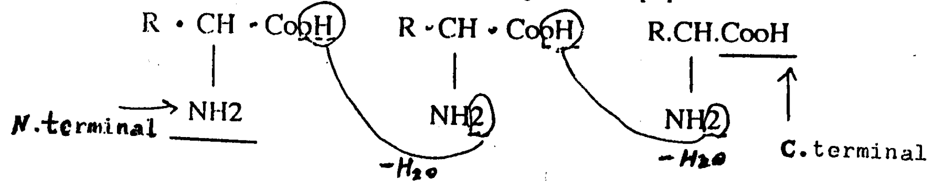
Peptide bonds are formed between the activated carboxyl group of amino acid and the amine group of other amino acid by losing one mole of water.

Biomedical Importance:

- Many hormones are peptide in nature, such as insulin (pancreas) oxytocin (post-pituitary), and vassopressin (post-pituitary).
- Certain antibiotics are peptide in nature containing L and D amino acids such as gramicidin and valinomycin.
- Some peptide hormones are synthetized by recombinant DNA technology producing the same structure and biological action of the native hormone.

Primary Structure of Peptides:

- The amino acid seqence (arrangement) in a peptide chain is referred as its primary structure.
- The number, kind, and linear sequence of an peptide determine its biological function as in hormones.
- In a peptide chain the N-terminal amino acid is always to the left side, and the C. terminal carboxyl end is at right of the peptide chain.



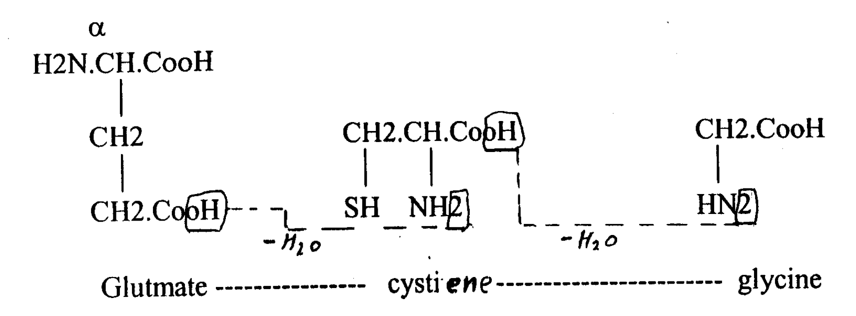
- Substitution of amino acid in sequence of primary structure of polypeptide may abolish its biological function, ad serious effects may arised as in sickle cell anaemia [valine instead of glutamic acid in position 6 of β chain globin].

Biologically Active Peptides:

Are peptides has biological functions:

(1) Glutathione:

Is tripeptide arised from glutamate, cystiene, and glycine through 2 peptide bonds.



Function:

1. Glutathione protect cell membrane from lysis; prevent haemolysis of RBCs, with aid of glutathione reductase via inhibition of the oxidizing agent H_2O_2.
2. Glutathione participates in metabolism of xenobiotics (it combines with toxins to render them non toxic compounds).
3. It inactivates insulin hormone.
4. Takes role in transport of amino acids across intestinal cells.

(2) Gramicidins: [containing 10 amin acids]:

Is decapeptide antibiotic produced by bacteria, it contains D & L amino acids.

** Cyclosporine: is cyclic natural colypeptides (11 A.A.) derived from fungi, is potent immunosuppressor for T-cells decreases rejection of transplanted organs and resistant of anticancer drugs.

(3) Oxytocin: [containing 9 amino acids]:

(produced by hypothalamus, transported by axons to post-pituitary):

Is hormone from post pitiutary, causes uterine coutraction, ejection of milk in females.

(4) Vasopressin: ***(Autidiuretic hormone, ADH), [containing 9 amino acids]:***

Produced in hypothalàmus, transoported by axons to post-pit.

Is secreted from post pituitary, it causes rise in blood pressure, and retention of water by kidneys.

(5) Bradykinin: [containing 9 amino acids]:

Librated from specific plasma proteins by proteolytic enzymes as trypsin, it causes relaxation of smooth muscles, vasodilation and hypotension.

(6) β. lipotropin: [containing 91 amino acids]:

Is polypeptide produced mainly from anterior pituitary, it acts as the precursor of β endorphin. It has minimal role in lipolysis, and fat mobilization.

(7) β. endorphin: [Thirty one amino acids]:

Arised from β. lipotropin in anterior pituitary, it acts as neurotransmitter or neuromedulator, has high analgesic power than morphine by 18-30 times, control pain perception.

•It binds to C.N.S opiate receptors. Its level may increased by regular aerobic ,prayer,or meditation. But gush increase occures in acute stress conditions as in accidants.

It has other sutypes, such as α endorphin (14), and γ endorphin (15) A.A.

- Increase endorphin levels prevent immune system inhibition resulting from depression of bereavement.
- Depressed people or alcoholics has low levels of β.endorphin.

(8) ACTH: [containing 39 amino acids]:

- Produced from antipituitary, it increased the synthesis and release of adrenal cortical steroids from supra renal cortex.

(9) Aspartam:

- Is dipeptides (Aspartic, A&Ph.alanin), is sweeter than sucrose 180 times, thermolabile at high temp.

Determination of the primary structure of peptides; through:

1. Separation of amino acids by rupture of peptide bonds via acid, alkaline or enzymatic hydrolysis.
2. identification of amino acid residues by chromatography, ion exchange column chromatography or by electrophoresis.

** Primary structure of peptide may be determined by automated technique by Edman, in which number, kind and sequence of amino acids are determined in one set.

Synthesis of Peptides:

Peptides can be synthesized by automated technique after determining its primary structure using insoluble resin particles.

Synthesized peptides are oxytocin, vasopressin, ACTH, and MSH.

III) PROTEINS

Definition:

Are nitrogenous organic compounds, which are present in all living cells whatever of animal or plant origin.

On protein hydrolysis it give rise to α amino acids.

Chemical Composition of Proteins:

- Proteins are composed of elements, such as:

Carbon 45-55%, hydrogen 6-8%, oxygen 19-25%, nitrogen 14-20% and sulphur 0-4%.

Biomedical Importance:

(1) Proteins are essential components of the cell protoplasm, enter in formation of cell enzymes required for different biochemical reactions.

(2) Proteins act as receptors and regulators at cell membranes for different hormones and compounds.

(3) Protein, such as Hb, act as carrier of oxygen to tissues and CO2 to lung. myoglobin acts as store of oxygen in muscles.

(4) Proteins, combine with nucleic acids (DNA & RNA) to form nucleoprotein for cell devision and protein biosynthesis.

(5) Serum proteins perform many biological functions [albumin, α_1, α_2, β, and γ globulins).

(6) **Blood clotting**: coagulation factors are protien in nature.

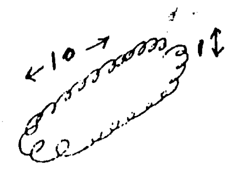
Classification of Proteins:

Proteins may be be classified to its overall shape into:

(1) Globular Proteins:

- In which the peptide chains are compact, folded, and coiled, ratios of length to breadth is 4 to 1, such as Hb, myoglobin and insulin.

(2) Fibrous Proteins:



- The peptide chains are elongeted and folded like a spiral, ratios of length to breadth is greater them 10-1, such as collagen, keratin of hair, myosin of muscles and silk fibroin of silk worm, which composed mainly from glycine with either alanine or serine.
- The chains may linked together by cystine strong bond as in keratin.

Proteins also, may be classified according to solubility:

(I) Simple Proteins:-

- On hydrolysis give rise to α amino acids only, they include:

(1) Albumins: (soluble in:water, saline dil. acids and alkali)

- Soluble in distilled water, coagulated by heat, present in serum, egg white, and milk; are first class proteins. ppt. by full saturation with Amn. SO_4, M.W 68.000 .

(2) Globulins: (soluble in :saline, dil acid&alkali).

- Soluble in saline, coagulated by heat, present in serum, egg yolk, milk; are first class proteins. ppt. by half saturation with Amn. SO_4, M.W 150.000 .

(3) Glutelins:

- Insoluble in water or saline, but soluble in acid or alkaline solutions, has glutin protein, rich in glutamic acid.
- Glutin may cause allergic diarrhea (sprue) in some patients.
- Glutelins are present in wheat and rice, gives elasticity of dough.

(4) Prolamines:

- Insoluble in water, soluble in alcohol, rich in proline, present in maize (lacking in tryptophan), barely (has tryptophan), and in rice.

(5) Protamines:

- Soluble in 70% ethanol, rich in basic amino acids (lysine, argenine), present in nucleoptroteins of fish.

(6) Histones: (Globins)

- Soluble in salt solutions, rich in basic amino acids (histadine and lysine), present in nucleoproteins of liver and spleen with DNA form the nucleosomes.

(7) Scleroproteins (hard proteins):

- Insoluble in water or diluted acids ad alkalies, rich in glycine, and proline, present in nails, horns, cartilages, bones and tendons (extracellular protective proteins).
- Scleroproteins are fibrous protein has supporting and protection functions.
- Other variants of scleroproteins are: collagen, elastin, keratin and reticulin.

**** Collagen**

- Present in Con.Tiss of skin, tendons, bones and blood vessels, form about 30% of total body proteins.
- Is formed of 3 polypeptide chains, each contains 1050 amino acids.
- Arised from fibroblasts (Con.Tiss cells).

(II) Conjugated Proteins:

On hydrolysis give rise to α amino acids, in addition to non protein radical such as: phosphoric acid, lipids, CHO, nucleic, metals and pigments.

(1) Phosphoprotiens:

- Are insoluble in water, but soluble in dilute alkalies.
- Phosphoric acid is attached to the hydroxyl group (-oH) of serine in the protein molecule.
- Phosphoproteins are first class protein, rich in tryptophan and methionine, so are lipotropic.
- Phosphoproteins are present in egg yolk and casein of milk.

(2) Lipoproteins:

- Are water soluble, in which the protein moiety surrounds the lipid part.
- Lipoproteins in blood carry lipids to body tissues for distribution and oxidation.
- Lipoproteins present in blood in the form of chylomicons, pre. β, β-lipoprotein, and alpha lipoproteins.

(3) Glycoproteins:

- Are proteins associated with sugars (galoctose, mannose, sialic acid, glucosamine, and may fucose).
- Glycoproteins are present in serum (soluble) as γ globulins or in cell membranes as receptors (insoluble).
- Glycoprotiens are present in two forms: i) N.linked;in which CHO radicle is linked to amide group of aspargine. ii)O.linked in which CHO is linked to OH group of serine or threonine.

4- Núcleoproteins:

* Are proteins (histones or protamins) associated with nucleic .
* present in D N A of the cell for cell devission and transfer of genetic characters , or in cytoplasm RNA for protein biosynthesis.

5-Metalloproteins:

*are proteins containing metal as prosthetic group:

(A) **Iron** : present in the forms of : ferrous

- Hemoproteins : are proteins with ferrous heme ring (Red colour) as Hb , myoglobin , cytochiames , and catalase.
- transferring : is transport iron in the plasma (B. globulin + ferric . OH)
- ferritin : is storagl form of iron , iutesrine ,spleen and bone marrow (apoferritin + ferric . OH)

(b) **Copper:**

-Ceruloplasmin : in plasma (α_2 globulin + CupricOH) it increased in hepatic cirrhosis.

- Hepatocuprein : present in liver.

-Cerebrocuprein : in brain, contains both cupper and zinc.

-Hemocuprein : in R.B.C.s of snake(bluish colour)

-Enzymes : Tyrosinase, Cytochrome oxidase and Ascorbic acid oxidase .

(C) **Zinc:**

present as crystals in insulin in β cells of pancrease

in some enzymes : Carbonic anhydrase, Carboxy peptidase .

Zinc has role in raising body immunity and promotes wound healing

(d) **Magnesium :**

in Chlorophyl of plants giving its green colour

in some enzymes : Phosphatase , Phosphorylase and kinases .

(E)**Mangancese :**

present in enzymes as Arginase and Choline estrase .

(6) **Chromoproteins:** (Coloured proteins)

are proteins containing pigments as prosthetic group, it is divided into :

1- **Metalochromoproteins :** proteins +Pigments +Metals

Hb: is Iron containing proteins (red colour)

Chlorophyll: in plants containing Magnesium (green colour)

Hemocuprein : its Heme containing copper (green bluish colour)

2-Non **Metalochromoproteins** : proteins +Pigments

* **Melanoproteins :** are black in colour, in Hair , in Skin and Iris
* **Flavoproteins :** are Yellowish in colour as FAD , FMN and amino acid oxidase
* **Carotenoid pigment:** Orange in colour present in Rhodopsins (Rods) and Iodopsins (Cones) in retina, also in Carotines of some plants.

(III) Derived Protein:

Are derived from hydrolysis of protein. Protein ----------> metaprotein -----------> proteose ---------> peptone ----------> polypeptides, and gelatin (obtained by prolonged hydrolysed of collagen in bones.)

Bonds In Protein Structure:

[By x ray crystallography] for protein crystals, protein structure is stabilized by 2 strong covalent bonds [peptide, and disulfide], and 3 weak bonds as (hydrogen, hydrophobe, and electrostatic (ionized) bonds).

(1) Peptide Bond:

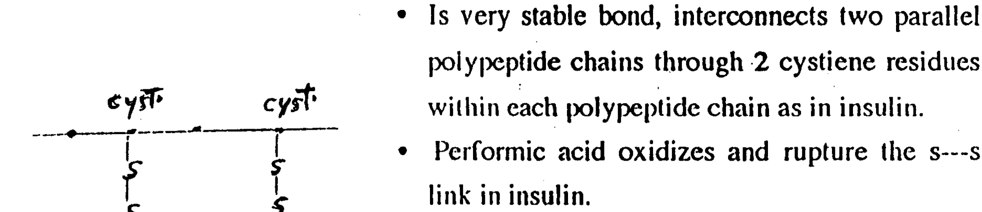
- L. α. amino acids in protein are linked together through the carboxyl group of one amino acid and the amine group of another amino acid, with release one mole of water.
- Peptide bond determines the primary structure of the same polypeptide chain.
- Biuret's reagent reacts with peptide bonds (2 or more) giving purple colour.

CH3.CH.CoOH (NH2) + CH3.CH.CooH (NH2) -----------> CH3. CH. CooH (NH–C(=O).CH.CH3 (NH2)) + H2O

$$CH_3.CH(NH_2).CO{-}OH + CH_3.CH(NH_2).COOH \longrightarrow CH_3.CH(NH_2).C(=O){-}{-}{-}NH.CH(CH_3).COOH + H_2O$$

Dipeptide

(2) Disulphide Bond:



- Is very stable bond, interconnects two parallel polypeptide chains through 2 cystiene residues within each polypeptide chain as in insulin.
- Performic acid oxidizes and rupture the s---s link in insulin.

(3) Hydrogen Bond:

- Binds two parcllel extended polypeptide chains through the carboxyl oxygen of one chain, and H. atom of (NH) of other chain.
- During denaturation of protein, the hydrogen and hydrophobic bonds are broken, not peptide or disulfide.

```
                      R3     ↓     R4
                      |            |
one chain — C — C — N — C — C — N—
            ||  |   |   ||  |   |
            O   H   H   O   H   H
H. bond  →  |   |   |   |   |   |
            H   H   O   H   H   O
            |   |   ||  |   |   ||
one chain — N — C — C — N — C — C —
                |     ↑     |
                R1  peptide R2
                     bond
```

(4) Hydrophobic Bond:

```
      R1 - - - - - - - - R2 - - - - - - - - R3
      |                  |                  |
N — CH — C — N — CH — C — N — CH — C
|        ||  |        ||  |        ||
H        O   H        O   H        O
```

- [The nonpolar side chains of neutral amino acids tend to associate in protein].

- Formed between the non polar hydrophobic side chains (R) of neutral amino acids, the bond lie deep within the molecule; the hydropholic (polar) part is assoicated with water and is found on the surface of the molecule.

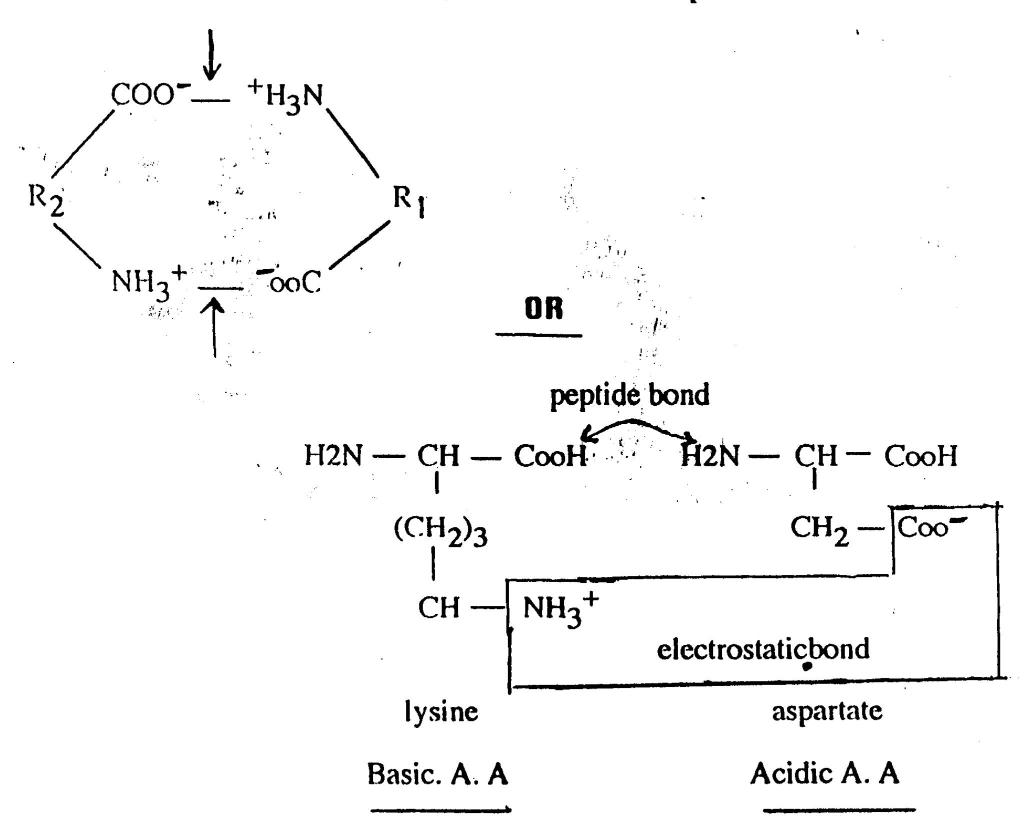
```
HooC — R — NH2
       |
H2N — R — CooH
```

OR

R, CH_2 —, CH_2 —, R

(5) Electrostatic; Ionic; Polar; or Salt Bond:

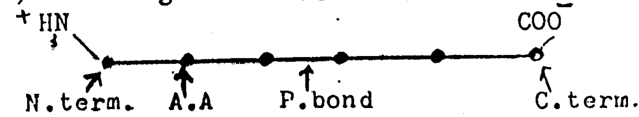
- This bond is formed between two opposite (+ve, -ve) charged groups in two amino acids, when they are both ionized [as in basic and acidic amino acids].
- The bond is important in binding basic proteins with other acidic macromolecules (DNA & RNA) to form the nucleoprotins.



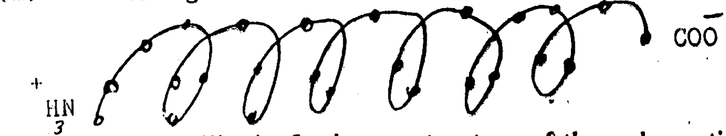
Orders or Protein Structure:

(1) Primary Structure:

- Is number, kind and linear seqence (arrangement) of amino acids in the polypeptide chain.
- The amino group (NH2) is to the left side and called N. terminal, the carboxyl group (-CooH) is to the right side and called C. terminal.

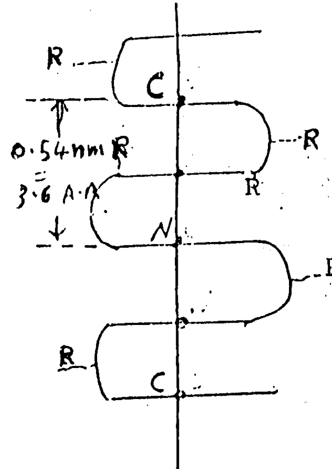


(2) Secondary Structure:

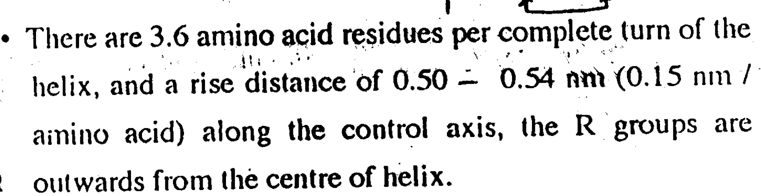


- Is folding (coiling) of primary structure of the polypeptide chain to form specific coiled (helix) structure, held together mainly by hydrogen bonds to stabilize it or by disulfide bond.
- The secondary structure of the polypeptide chain can be exist in the form of a coil or helix, named α helix.

i) The α helix form:



- In which the peptide chains may present in highly ordered form and stabilized by large numbers of hydrogen bonds between its peptide residues.



- There are 3.6 amino acid residues per complete turn of the helix, and a rise distance of 0.50 – 0.54 nm (0.15 nm / amino acid) along the control axis, the R groups are outwards from the centre of helix.
- The α helix form may be present in both fibrous (keratin) protein, and globular proteins (albumin & myoglobalins).

ii) The β-Pleated Form:

- In another form of secondary structure, where the peptide chains are fully extended (not coiled), and stabilized also by the hydrogen bonds.
- When adjacent peptide chains in β pleated sheet run in opposite direction (N. terminal to C. terminal) and vice versa are called aniparallel.
- If run in the same direction are called parallel.
- The β-pleated sheet form is found mainly in fibrous proteins as hair kertin.

iii) Randum Coil Form:

- Some regions in proteins not identified as α helix or β-pleated sheet, but present in randum coil form.
- Randum coils are of equal importance as in α helix or β.pleated sheet.

3) Tertiary Structure:

- The single polypeptide chain has its own primary and secondary structure, is further coiled and arranged in relation to other fibers or connective tissues to give the overall shape of the protein molecule (globular or fibrous) through the hydrogen bonds.

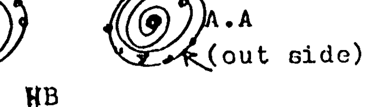
4) Quaternary Structure:

- Occurs in proteins which has several polypeptide chains (subunits), and each chain has its specific primary secondary and tertiary structures.
- Quaternary structures of these peptide chains is the spatial arrangement or relationship between these subunits to form the whole molecule.
- The forces stabilizing that structure is hydrogen or electrostatic bonds, as in LDH (5 subunits) or Hb [4 subunits].

Example:

Hb:

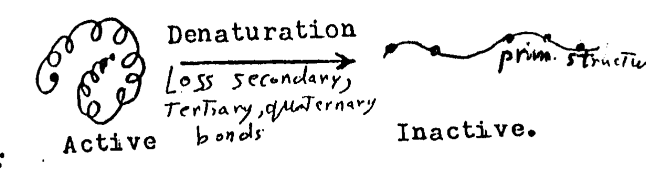
- Has four subunits peptide chains (2 α. chains; each has 141 amino acid residues, and 2 β chains; each has 146 amino acid residues).
- Every chain has its own primary, secondary, and tertiary structure.
- These subunits associate together to form the quaternary structure of Hb molecule.
- Hb is globular allosteric protein.



Denaturation of Proteins:

Definition:

- Is disorder of spatial arrangement of the protein from the native ordered structure of the protein; or loss of secondary, tertiary, and quaternory structures due to breaking of hydrogen and hydrophobic bonds.
- The reaction is mostly irreversable.



Properties of Denatured Proteins:

- Loss of biological, physical, and chemical properties.
- The proteins tend to aggregate and easily precipitate from solution.

Factors causing denaturation:

i) Physical:

- Heat, Freezing, X Ray, U. V. Rays, Ultrasound.

ii) Chemical:

- Extremes of pH, and Organic Slovents.

iii) Biological:

- As proteolytic enzymes (denaturation occures before hydrolysis).

Myoglobin & Hemoglobin Proteins

Definition:

Myoglobin and haemoglobin are compound metalloprotein, containing ferrous iron (Fe^{++}) as a prosthetic group. Hb is present in red cells, but myoglobins in cytoplasm of red skeletal muscles and heart cells.

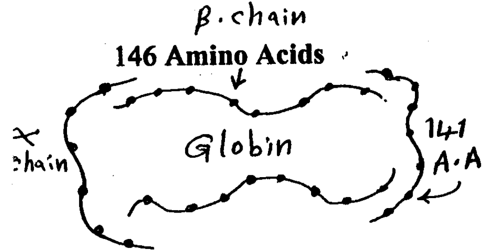
Biomedical Imortance:

1. Hb functions in oxygen binding and transport O_2 from lung to body tissues.
2. Hb can carry CO_2 from tissues to lung.
3. Myoglobins in red muscle tissue bind and store oxygen to release it to muscles in cases of muscular exercise to aid in ATP synthesis for muscles, in acute myocardial infarction, myoglobin is released into circulation in first 4 hours.
4. Hb acts as important blood buffer [$\frac{Hb}{K.\ Hb}$ and $\frac{Hb\ O_2}{K.Hb.O_2}$].
5. Genetic defects in sequence or synthesis of Hb peptide chain result in blood haemolytic anemia such as sickle cell anemia ad thalassemias respectively.

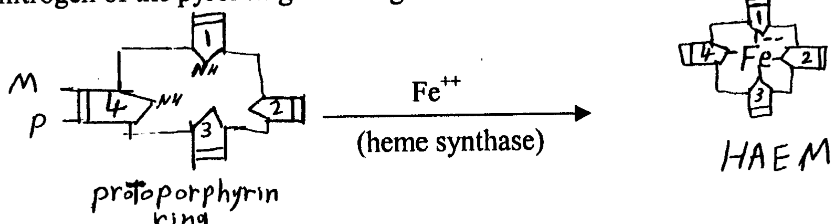
Structures of Myoglobin & Hemoglobin:

(1) Hb:

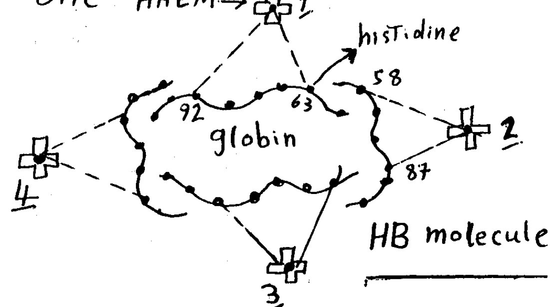
- Hb is red metallochromoprotein containing iron as haem.
- Its protein is rich in histones and is globular in structure, form 95% of whole Hb in RBcs.
- Its haem part form 5% of whole Hb.



- Its globular protein consists of four polypeptide chains; 2 α similar chains (141 amino acids) and two similar β chain (146 amino acids), all are in form of tetromer (globin part).
- The haem part consists of four pyrrols rings linked together by 4 methylene bridges, forming the porphyrin ring.
- The prophyrin can conjugate with ferrous iron (Fe^{++}) through the four nitrogen of the pyrol rings forming the haem.



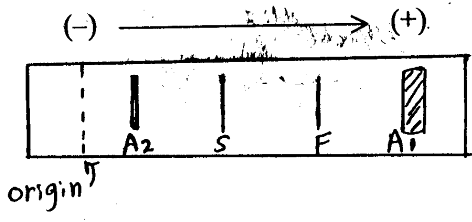
- Each chain of the globin part is attached to one haem atom forming the Hb with its quaternary structure.
- Hb can bind to $4O_2$ molecules (has 4 peptides chains).
- The haem atom is attached to the histidine residues of the Hb chains (to nitrogen atom of the imidazole ring).
- Normal Hb in adults is 14-16gm/dl, in whole blood 750gm; 6-25gm are produced and destroyed/day (changed over).



- Normal adult Hb consists of:
  Hb A1:98% (2α + 2β)
  Hb A2:2% (2α + 2δ) two delta chains in which argenine replaces Gly in position 16.

** Foetal (HbF): lysine replaces glutamate in position 6 of β chain γ → chain; ($\alpha_2 \gamma_2$).

** Hb F: forms 60% of Hb at birth, but in adults is less than 1%.



(II) Myoblogin:-

- Myoglobin consists of one haem group only; and single polypeptide chain of mol. Weight 17,000 and 153 amino acid residues, divided into 8 segments (A,B,C,...H).
- Its protein is globular in shape, has no quaternary structure.
- Myoglobin is present in red muscle tissue and store oxygen to release it to muscular mitochondria for ATP generation during muscular exercise.
- Myoglobin binds only to one O_2 molecule (has one polypeptide chain); monomeric.

Hb Derivatives:

(1) <u>Oxy Hb (Hb O_2)</u>: (Reduced Oxy Hb):

- Its oxygen saturation is 97% of arterial blood. The ferrous iron (Fe^{++}) leaves one imidozale of histi-dine residue, and becomes loosely attached to O_2, Hb molecule attaches to four O_2 molecules, but myoglobin attaches to one O_2 only.
- Its Hb is light red in colour.

(2) <u>Reduced Hb (Hb)</u>: (Reduced Oxy Hb):

- Is 40-70% of venous blood, its ferrous (Fe^{++}) iron is attached to the imidozale groups of the two histidines in the polypeptide chain.
- Its Hb is deep red in colour.

(3) Methemoglobin: (Hb.M); ferri. HB:-

- Its iron oxidized to ferric (Fe^{+++}) by ferricyanide, chlorates, ozone, nitrates, which cannot (binds) carry oxygen (useless), and is named HbM, cyanosis is developed (loss of oxygen capacity).
- Hb.M is brownish in colour, if increased ⟶ dusty skin.
- In inhireted Hb.M; histidine in position 58 (chain) or 63 (chain) are replaced by tyrosine in the two chains only.

(4) Carboxy Hb:-

- Arised due to exposure of blood to CO, which has high affinity to CO, 210 times than O_2, so Hb is not oxygenated, CO is very toxic.
- CO.Hb is red chairy in colour.

Other Variants (Types) of Hb:

(1) Hb S: ($\alpha_2\beta^s{}_2$):

- Is abnormal Hb due to mutation affects the sequence of amino acids; polar glu.A. is replaced by (non polar) valine in position 6 of β chain resulting in haemolytic anacia.
- HbS, may be homozygous (HbS/HbS); inherited from two parents, have severe symptoms.
- HbS, may be heterozygous (HbA/HbS), inherited from one parent only, have mild symptoms, this condition is called also sickle cell trait.
- In sickle cell trait (HbAs); offers protection against malaria falciparum due to phagocytosis of lysed red sickled cells which contain the parasites.

(2) β. Thalassemia:

- There is suppression in synthesis of β^o chain, and compensatory increase in γ or α chains, resulting in anaemia, HbF is increased.
- Excess alpha chains are unstable and precipitate ⟶ damage & premature destruction of red cells (sever hemolytic anemia).

(i) Thalassemia major; Cooley's anaermia:

- Is homozygous, its normal HbA_1, is reduced to 5-35%, and abnormal HbF is increased to 60-95%, serum iron is raised, clinical condition is severe.

(ii) Thalassemia minor ; Cooly 's trait anaemia :

* Is heterozygus , its normal HbA_1, is slightly reduced to 84 - 94 % abnormal H b F is slightly raised 2 - 5 % , the condition is mild .

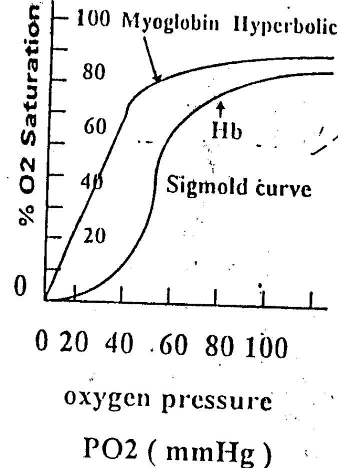
Function of Hb & myoglobin in carrying oxygen :

OR : Oxygen saturation (dissociation) curves of Hb & myoglobin (uptake and release of O_2) .

OR : Why Hb is suitable for O_2 transport to tissues, and myoglobin is suitable to store oxygen for muscles only ?

(1) Myoglobin :

* Found only in cytoplasm of red skeletal muscles, and in heart muscle .



* It storages and transport O_2 from cell membrane to mitochondria in muscles .
* It can bind to O_2 in similar as Hb, but is unable unable to release it, except under very low oxygen tension (5 mm Hg) = po_2 (partial pressure)
* PO2 at lung is 100 mm/Hg, at venous blood (peripheral tissue 40 - 50 mm / Hg, and at active muscles 20 mm / Hg) .
* Therefore, myoglobin is uneffective in releasing O_2 form lung to peripheral tissues, except in severe muscular exercise where PO2 of muscles is lowered to about 5 mm/Hg, here myoglobin releases its O2 to the mitochondria of muscles to provid its ATP .
* So, if PO2 (oxygen conc)is plotted against oxygen saturation % (is quantity % of O_2 binding with Hb, compared with total amount of oxygen which could combine with Hb), the curve of myoglobin is hyperbolic .

* Myoglobin cannot release its bound O2 at PO2 more than 20mm/Hg, so is suitable for muscles only .

** clinical significance :

1 - * Increased serum or urinary myoglobin may used as indicaton of early acute myocardial infarction than that of Cpk .

* peak value of serum myoglobin (in first few hours) correlates with the size of the carcdiac infarction .

* peak value of Cpk starts late after 4 hours .

2 - * Also serum or urinary myoglobin (myoglobinuria) is increased in muscular or crush injuries .

* the myoglobin is small in size and rapidly cleared through the kidneys in which may be sufficient to cause renal tubules obstruction , acute renal failure and death .

Detection of myoglobin :

Urine :

- myoglobinuria is dark in colour as cola or coffee .
- myoglobinuria and hemoglobinuria can be precipitated by sulphosalicylic acid reagent 20 % , but not by heating and acetic acid (give false negative result) .

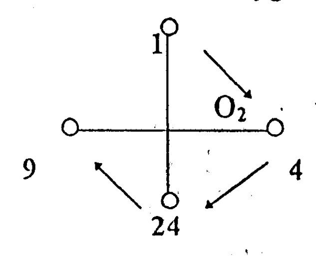
* Then by electrophoresis to differentiate between myoglobin and Hb .

Serum :

Serum myoglobin can be detected by the radio immune procedure or Elisa technique for rapid detection of cardiac infarction .

(2) Hemoglobin :

* Its curve is sigmoid rather than hyperbola, because Hb has 4 sites for binding of 4 oxygen moles (allosteric protein, has quaternary structure).
* At high PO2 in lung the binding of one mole of O2 produce confromational change by rupture of salt bonds between the subtypes chains and facilitates binding of next O2 molecule more readily due to presence of 4 sites of O2 binding, rather than one site in myoglobin.
* The ratio of oxygen bind to Hb is 1 : 4 : 24 : 9



So its O_2 saturation(bound to Hb) is high, which is suitable for fully loading of O_2 at lung, and in maxium release of O_2 at peripheral tissue than that of myoglobin.

HB

** The Hb - oxygen dissociation curve is as such at the normal pH (7.4) of the blood , where PCO_2 is 40 mm Hg, however the curve may be shifted to right or to left.

** Shift to right :

- results in greater release of O_2 from HbO_2 at a given oxygen tension ; or decrease affinity of Hb for oxygen.
- Shift of Hb - oxygen dissociation curve to right by increose PCO_2 tension is termed the Bohr effect (due to increose H . ion concentration derived from increase H_2CO_3 generation).
- Causes :

  1 - Increase blood acidity (↓pH).
  2 - Increase PCO_2 tension (above 40 mmHg).
  3 - Increase Temperature.
  4 - Increase 2 , 3 diphosphoglycerate in RB cs.

** Shift to left :

* Increase affinity of Hb for oxygen , results in decrease release of O_2 from Hb at a given oxygen tension .

** Composition of inhaled air : (in a clear cool day)

O_2 : 20 .8 % , N : 78.6 % , CO_2 : 0 . 04 % , H_2O : 0.5 %

* Inspired oxygen diffuses from alveali to blood $\longrightarrow$ HbO_2 .

* Nitrogen is inert gas , does uot bind to Hb .

* 98 % of inspired oxygen is carried on Hb by physical diffusion

* forming the HbO_2 .

** composition of expired air :

O_2 : 15.7 % , N : 74.9 % , CO_2 : 5 % , H2O : 6.2 %

•• about 5% of inhaled oxygen is replaced by CO_2 in expired air .

Function of Hb in transporting CO_2 and protons from peripheral tissues to the lung :

* CO_2 generated by the kreb's cycle , is absorbed in the blood and diffuses to RBcs where it catalyzed to H_2CO_3 by the carbonic anhydrase enzyme (present in membrane of RBcs) .

$$2\,CO_2 + 2H_2O \xleftrightarrow[\text{anhydrase in RBcs}]{\text{carbonic}} 2\,H_2CO_2$$

* H_2CO_3 rapidly dissociates into bicarbonate and proton .

$$2H_2CO_2 \xleftrightarrow{\text{SPONTANOUS}} 2H^+ + 2HCO^-_3$$

* To avoid excess acidity of the blood, Hb acts as buffer and binds to 2 proton for every 4 oxygen molecules lost ($Hb . 2H^+$)

* In the lung , the reverse occures ; $4O_2$ molecules bind to deoxygenated Hb, and the two protons are released .

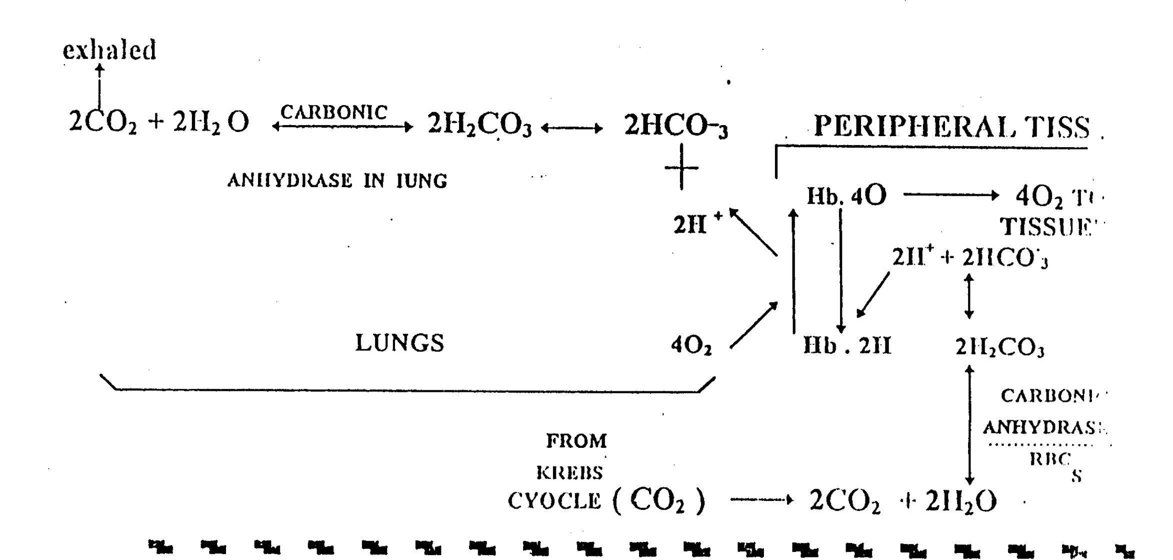
* The two protons bind with bicarbonate forming H_2CO_3.

$$2H^+ + 2HCO^-_3 \xleftrightarrow{\text{SPONTANOUS}} 2\,H_2CO_3$$

* In lung cabonic acid is catalyzed by carbonic anhydrase, and CO_2 is formed, which is exhaled .

$$2H_2CO_3 \xleftrightarrow[\text{anhydrase (RBcs in lung)}]{\text{carbonic}} 2CO_2 \text{ (exhaled)} + 2H_2O$$

* Thus, binding of $4O_2$ to the deoxygenated Hb forces the exhalation of CO_2, the phenomena is called Bohr effect .



LIPIDS

Are organic heterogeneous compounds, insoluble in water, but soluble in fat solvents such as (ether, benzene and chloroform).

They include fats.oils, waxes, steroids, and fat sol. vitamins. Lipids are esters of fatty acids(R.COOH), with alcohols(R.OH).

❖ Biomedical Importance:

- Lipids serve as efficient source of energy [one mole of CHO → 38ATP, while one mole of palmitic acid → 129 ATP].
- Enter in structure of all cell membranes; (phospholipid bilayer, and cholesterol) for integration of cell membrane.
- Act as thermal isolator around internal organs.
- Combination of fat and proteins (lipoproteins) act as transporter of lipids in the blood. to be oxidized inside the cells.
- Study of lipids is important in obesity, atherosclerosis, while unsaturated fatty acids are essential for health.
- Degradation of fatty acids give rise to acetyl CoA which is the base of cholesterol formation, ketone bodies, and provide much of energy.

❖ Classification:

(A) Simple Lipids:

Are esters of fatty acids with alcohols.

It includes:

1) **Neutral fats**: are esters of 3 fatty acids with glycerol (trihydric alcohol), they are also called triacylglycerol, at room temperature are called fats if in hard state, or called oils if in liquid state.

2) **Waxes**: are esters of one fatty acid, with monohydric alcohol has carbons higher than alcohol as (mericyl alcohol $C_{30}H_{61}.OH$ + palmitic acid→ bee wax).

Waxes cannot be utilized by body, not attacked by bacteria or lipase, so never become rancid.

($R.COOH + C_{30}H_{61}.OH \longrightarrow C_{30}H_{61}O\text{-}OCR$) Wax.

(B) Compound Lipids:

Are esters of fatty acids with alcohols in addition to other groups as:

Phosphoric acid residue → phospholipids. (cell membr., lung, HDL)

Carbohydrate residue → glycolipids (Nervous Tiss).

Proteins residue → lipoproteins (In blood).

(C) Derived lipids:

Are derived from the above groups by hydrolysis such as fatty acids, glycerol, steroids, in addition to ketone bodies, fat soluble vitamins, steroidal hormones, and carotenoids [carotene pigments].

CHEMISTRY OF SIMPLE LIPIDS AND ITS COMPONENTS [GLYCEROL & FATTY ACIDS]

i) Glycerol (glycerine):

- Is trihydric alcohol, colourless, odourless, sweet in taste, hygroscopic [miscible with water], used in vanishing creams for dry skin.
- Glycerol is formed by special fermentation of glucose, in body can give rise to glucose.
- Glycerol can be detected with acrolein test if heated with H_2SO_4 which dehydrates glycerol producing compound with irritating odour (acrolein).

CH_2–OH
CH–OH GLYCEROL
CH_2–OH

ii) Fatty Acids:

- Are monocarboxylic acids, obtained by hydrolysis of fats.
- Occur in straight chains, with an even number of carbon atoms.
- Fatty acid chain may be: $(CH_3.CH_2.CH_2.......COOH)$
  a) Saturated (containing no double bonds), or
  b) Unsaturated (containing one or more double bonds).
- Fatty acids are insoluble with water, but soluble in non polar solvents (benzene and ether), has low specific gravity than water, so float on surface.

(A)Saturated Fatty Acids: (has no double bonds)

Composition:

Saturated fatty acids are composed of straight chain of hydrocarbons with carboxyl group at the right side, and is

numbered as C_1, next is C_2 which named α carbon, C_3 is the β carbon,

- The left end methyl carbon is ω omega carbon, and in between $-CH_2-$groups are added in even numbers. Sat.f.A end by suffix (-anoic);as palmitic.A(16C)named hexadecanoic (hexa=6,deca= 10).

$$\overset{\omega}{CH_3}.CH_2..........\overset{\gamma}{CH_2}.\overset{\beta}{CH_2}.\overset{\alpha}{CH_2}.COOH$$

methyl end (omega) 4 3 2 1

- Fatty acid mole consists of:
  - i) Hydrophobic non polar part (methyl and hydrocarbons parts; forming the hydrophobic tail).
  - ii) The other hydrophilic polar part (the carboxyl group) forms the polar head group.
- Lipids float on surface of water (less specific gravity) where the polar head group is facing to water, while the non polar tail part is outside facing air (amphipathic property).

$CH_3.CH.CH..............CH$

Hydrocarbon non polar tail — polar head group

$$CH_3.CH_2.CH_2...............CH_2.CH_2-C\begin{smallmatrix}\nearrow O \\ \searrow O^- \end{smallmatrix}$$

	Sat. F.A.	C Atoms	Comments
Short Chains	Acetic	2	Major end product of CHO metabolism
	Butyric	4	In certain fats as butter
	Caproic	6	In some animal fats
	Caprylic	8	In some fats of plant origin
	Capric	10	
Long Chains	Lauric	12	Coconut oil, laurels, cinnamon, palm kernel
	Myristic	14	Nutmeg, coconut oil, myrtles
	Palmitic	16	Common in al animal (including humans) and plant fats
	Stearic	18	
	Arachidic	20	Peanut oils (arachis)
	Behenic	22	Plant seeds
	Lignocervic	24	Cerebrosides & peanut oil

Importance:

- Rich source of energy.

- Excess fatty acids can be stored in the body as subcutaneous or depot fat; its excess causes obesity.
- Its degradation can give rise to acetyl CoA which is precursor of cholesterol biosynthesis, which in excess can cause atherosclerosis and affect the health.

***(B) Unsaturated fatty acids*:**

Has one or more double bond,
according to number of double bonds are classified into:

i) Monounsaturated (monoenoic, monoethenoid):

Has one double bond, non essential, **synthetized in the body.**

a- palmitoleic: (16:1, Δ^9, ω^7): in all fats = Hexadecenoic

16:1 = fatty acid with 16 carbon atoms, and one double bond.

Δ^9 = the position of double bond at delta (9) between C_9, and C_{10} from the carboxyl end.

ω^7 = the position of double bond on C_7 from the methyl omega side on the left side.

$$\overset{\omega}{CH_3}-(CH_2)_5.CH=CH-(CH_2)_7-COOH$$

b- Oleic acid: (18:1, Δ^9, ω^9)

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOH$$

c- Nervonic acid: (24:1, Δ^{15}, ω^9)

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_{13}-COOH$$

Nervonic acid present in glycolipids (cerebrosides)

**Unsat.F.A end by suffix (-enoic); as oleic acid(C18), named octadecenoic acid(octa=8, deca=10).

Unsaturated fatty acids.

Number of C Atoms and Position of Double Bonds	Series	Common Name	Systematic Name	Occurrence
			Monoenoic acids (one double bond)	
16:1;9	ω7	Palmitoleic	*cis*-9-Hexadecenoic	In nearly all fats. olive oil
18:1;9	ω9	Oleic	*cis*-9-Octadecenoic	Possibly the most common fatty acid in natural fats. olive oil
18:1;9	ω9	Elaidic	*trans*-9-Octadecenoic	Hydrogenated and ruminant fats.
18:1;11	ω7	Vaccenic	*cis*-11-Octadecenoic	Synthesized by bacteria.
22:1;13	ω9	Erucic	*cis*-13-Docosenoic	Rape and mustard seed oils.
			Dienoic acids (2 double bonds)	
18:2;9,12	ω6	Linoleic	all-*cis*-9,12-Octadecadienoic	Corn, peanut, cottonseed, soybean, and many plant oils.
			Trienoic acids (3 double bonds)	
18:3;6,9,12	ω6	γ-Linolenic	all-*cis*-6,9,12-Octadecatrienoic	Some plants, eg, oil of evening primrose; minor fatty acid in animals.
18:3;9,12,15	ω3	α-Linolenic	all-*cis*-9,12,15-Octadecatrienoic	Frequently found with linoleic acid but particularly in linseed oil.
			Tetraenoic acids (4 double bonds)	
20:4;5,8,11,14	ω6	Arachidonic	all-*cis*-5,8,11,14-Eicosatetraenoic	Found with linoleic acid particularly in peanut oil; important component of phospholipids in animals.
			Pentaenoic acids (5 double bonds)	
20:5;5,8,11,14,17	ω3	Timnodonic	all-*cis*-5,8,11,14,17-Eicosapentaenoic	Important component of fish oils, eg, cod liver oil.
22:5;7,10,13,16,19	ω3	Clupanodonic	all-*cis*-7,10,13,16,19-Docosapentaenoic	Fish oils, phospholipids in brain.
			Hexaenoic acids (6 double bonds)	
22:6;4,7,10,13,16,19	ω3	Cervonic	all-*cis*-4,7,10,13,16,19-Docosahexaenoic	Fish oils, phospholipids in brain.

 (NOT SYNTHETIZED IN BODY)

ii) Polyunsaturated (polyethenoid, polyenoic, essential fatty acids) :

a- Linoleic; (18:2, Δ9,12, ω6) has two double bonds.

$CH_3-(CH_2)_3-(CH_2-CH=CH)_2-(CH_2)_7.\ COOH$

*Can gives ise to arachidonic.A in the body by desaturation -2H→linolenic, then by chain elongation (+2C, -2H) → arachidonic.A

b- Linolenic: (18:3, $\Delta^{9,12,15}$, ω^3); three double bonds

$CH_3-(CH_2-CH=CH)_3-(CH_2)_7.\ COOH$

, present in: linseed oil, and with linoleic acid.

**Linoleic& α.linolenic should supplied in diet for complete nutrition ;are the only true essential.fatty.acids .

c- Arachidonic acid: (20:4, $\Delta^{5,8,11,14}$, ω^6), four double bonds.

present in: peanut oil, is component of phospholipids in animals.

d- Clupanodonic acid: (22:5, $\Delta^{7,10,13,16,19}$, ω^3) five double bonds.

Present in fish oils, phospholipids in brain.

iii) Eicosanoids: C20=(Eicosa.tetra.enoic):

Derived from polyunsaturated fatty acids;arachidonic after cyclization at its center to form the cyclopentan ring.

.Are powerfull hormone like molecule. act locally on smooth muscles.

.Present in every cell,but discovered orginally in prostate.

-Eicosanoids include:

I) Prostanoids:-

a) prostaglandins: (PG; A,B,D,E,F,H,I)

.Has local hormonal like action.

PG F: contraction of uterus and intestine,vasoconstrict.

PG E: smooth muscle relaxants,vasodilator.

b) Prostacyclins; PGI :

.Has additional ring between C_6 and C_9 .

.Is vasodilator,inhibit platelets aggregation.

c) Thromboxanes; TX:

.Is cyclic compound.,has oxane ring with oxygen.

.It stimulates platelets aggregation.

POLY.UNSAT.ARACHIDONIC.ACID
(eicosanoic; C20)

Arachidonic acid :

CH_3 20 14 11 8 5 1 COOH

cyclization at mid chain

Prostanoic acid :

9 8 6 5 1 COOH 10 11 12 14 15 CH_3 20

$2O_2$

PROSTAGLANDIN SYNTHASE

ketone group

cyclopentane ring

Prostaglandin :

O 9 8 10 6 5 4 1 COOH CH_3 20 12 13 14 15 17 19 OH OH

PGE₂

PGE	PGF α	TXA
.sol.in ether	.sol.in phosphate buffer	.has oxane ring

O, 9, 8, 10, 11, 12, R_1, R_2, OH — PGE

OH, 9, 8, 10, 11, 12, R_1, R_2, OH — PGF α

O, O, R_1, R_2 — TXA

II) Leukotriens;LT:

Are acyclic compounds,present in leucocytes,platelets, and mast cells,act as mediator in chemotaxis(kill organism) by lysozomal enzymes.

- *Importance of unsaturated fatty acids*:

i) *Monounsaturated fatty acids*:

Acts as protection against coronary heart diseases via increasing amounts of HDL. [Useful type of lipoprotein]

Monounsaturated fatty acids are present in olive oil, walnut, and peanut.

ii) Polyunsaturated fatty acids:

Protect against atherosclerosis and coronary heart diseases by decreasing cholesterol & LDL. (bad type of lipoprotein)

- *Sources of polyunsaturated fatty acids*:

Safflower oils, sunflower oil, corn oil, Soya bean oil, linseed oil, cotton seed oil, and fish oils which contain particularly the ω^3 polyunsaturated fatty acids.

- ω^3 :in linolenic;linseed oil, and fish oil.
- ω^6 :linoleic;corn oil,cotton oil,beanut oil(arachidonic.A).

Isomerism of unsaturated fatty acids:

Depends upon orientation of atoms around the double bond, so two forms of unsaturated fatty acids are arised:

1) CiS unsaturated fatty acid:

In which the H, H groups around the double bond at the same side

CIS, OLeic acid :

$$CH_3CH_2 - - - C(H) = C(H) - CH_2 \cdots COO^-$$

CiS form (isomer) present naturally in mammals and oils, so CiS isomer is beneficial type.

2) **Trans isomer**

In which the H,H groups are on the opposite side of the double bond

Trans , elaidic acid :

$$CH_3 \cdot CH_2 - - - C(H) = C(H) - CH_2 \cdots COO^-$$

- Present naturally in small amounts in ruminants via effect of microorganism on fat.
- Also present in larger amounts in hydrogenated vegetable oils (margarine).
- They metabolized like saturated fatty acids, so increase cholesterol & LDL and decrease HDL, also may impair metabolism of essential fatty acids.

Properties of Fatty acids:

- Physical properties:

1- **Solubility**:

- Short chains fatty acids (up to C_6) are soluble in water, as butyric and caproic fatty acids, but long chains are water insoluble but soluble in fat solvents.

2- Melting point:

- Short chains or unsaturated fatty acids are liquid at room temperature.
- Long chains or saturated fatty acids are solid at room temperature.

Chemical properties:

1- Salt formation (soap)

Fatty acids form soaps (salts) with alkalie as NaOH, KOH, $Ca(OH)_2$.

$$R.COOH + K.OH \rightarrow R.COO.K + H_2O$$

[R.COO.K is potassium salt of soap]

- Sodium and potassium salts are soluble in water
- Calcium and magnesium salts are insoluble in water.
- Water containing calcium or magnesium ions is called hard water due to replacement of hard water ions to sodium or potassium present in soap.
- But detergents are soluble in hard water.

2- Ester formation:

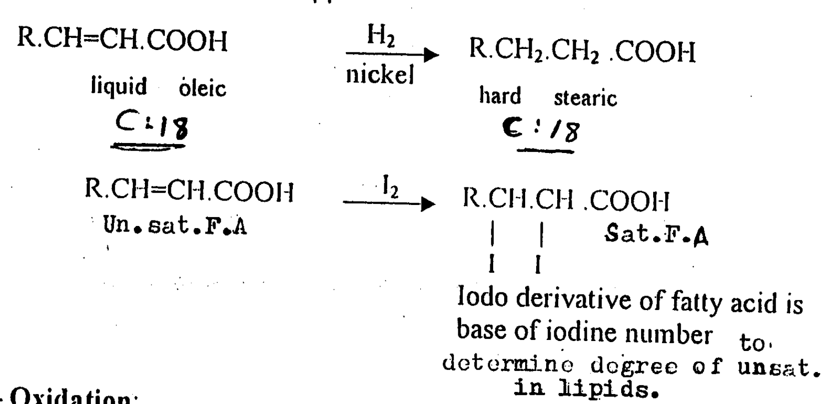
- Carboxyl group of F.A form esters with (OH) group of alcohol.

$$R.COOH + HO.R \rightarrow R.COO.R + H_2O \text{ [ester]}$$

- If with glycerol → neutral fat
- If with higher alcohol → wax

3- Hydrogenation or halogenation of unsaturated double bond:

- Hydrogen is added in presence of catalyst as nickel producing the corresponding saturated fatty acid (margarine)



4- **Oxidation**:

- Unsaturated fatty acids are oxidized slowly in air, but it proceeds rapidly in presence of ozone (O_3) giving rise to fatty acid peroxide, and then broken into shorter chain aldehydes.

$CH_2.CH{=}CH.CH_2 \xrightarrow{O_3}$ (Oleic.A) $\rightarrow$ oleic peroxide (ozonide ring: $CH_2\text{-}CH$ and $CH.CH_2$ bridged by O–O–O)

$\downarrow H_2O$

Aldehydes: $-CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}H + H\text{-}\overset{O}{\overset{\|}{C}}.CH_2- + H_2O_2$

(A) Neutral Fats (Triacylglycerols):

Are esters of glycerol and three fatty acids (mostly mixed).

Sources:

1. Animal source: butter, lards and mutton
2. Plant source: cotton seed oils, olive oil, corn oil, sunflower oil, and linseed oil.

3. Marine oils: cod liver oil, shark, and halibut liver oils (rich in vit. A& D) and ω^3 polyunsaturated fatty acid.

Structure:

$CH_2.OH$ | $CH.OH$ | $CH_2.OH$	+	$HOOC.R_1$ $HOOC.R_2$ $HOOC.R_3$	$\xrightarrow{-3H_2O}$	$CH_2\text{-}OOC\text{-}R_1$ | $CHOOC\text{-}R_2$ | $CH_2\text{-}OOC\text{-}R_3$
Glycerol		*3 F.A*		*neutral fat*

Physical properties:

1) *Solubility*:

All triacylglycerols are insoluble in water, but soluble in fat solvents.

2) *Melting point*:

- Neutral fats rich in unsaturated F.A. are liquid at room temp. and called oils.

3) Specific gravity: is less than 1, so float on water, so fatty swimmers are better than thin ones.

- Neutral fats rich in saturated fatty acids are solid at room temp. and called fats.

4) All give positive greese stain test.

Chemical properties:

1) *Acrolein test*:

All triacylglycerols give positive acrolein test due to presence of glycerol.

2) Hydrolysis

Is breakdown of fat in presence of water, hydrolysis may be:

- Enzymatic (lipase): triacylglycerol $\xrightarrow[H_2O]{\text{lipase}}$ glycerol + 3 F.A

- Alkalae: triacylglycerol $\xrightarrow{3NaOH}$ glycerol + 3.R.COONa (soap).

3) Hydrogenation:

Liquid oils in presence of hydrogen gas and nickel as catalyst give rise to hard saturated margarine, the reaction carried out in high temp.

4) Oxidation:

i- Drying oils:

Oils rich in unsaturated F.A like linseed oil, when exposed to oxygen of air, they changed into dry hard water proof material due to formation of peroxides.

```
 H   H          H  H
 |   |          |  |
-C = C-  --O2-->  -C--C-
                  |  |
                  O--O
```

ii- Rancidity:

- Is change in odour and taste of oils & fats due to exposure to bacteria, moisture or oxygen.

  a- Hydrolytic rancidity:

Mostly occurs in solid fat as butter in presence of water and bacterial enzymes (lipo-oxygenase), yielding glycerol and fatty acids.

b- Oxidative:

Mostly in liquid oils due to oxidation of the double bond giving rise to peroxides, which may induce, cancer in the cells.

c- Ketonic:

Is change of odour and taste of oils due to oxygen.

$$R.CH{=}CH.COOH \xrightarrow{O} R.\overset{\overset{\displaystyle O}{\|}}{C}.CH_2COOH$$

- *Detection of rancidity*:

By copper acetate test: which detects the free hydrolysed fatty acids.

- *Prevention of rancidity*:

By addition of antioxidants as vit. E, vit C, urates. Also avoid of moisture, oxygen, and heat.

Identification and separation of lipids in biological materials:

- Lipids are first extracted by fat solvents from the biological materials.
- Then identified by thin layer chromatography and detected by iodine vapour.

- The gas liquid chromatography is more sensitive technique and needs very small amounts of lipids.

(B) Compound (conjugated) lipids

- Are esters of fatty acids with alcohol (glycerol) in addition to other non lipid groups as phosphoric acid → phospholipids, carbohydrates → glycolipids, and proteins → lipoproteins.

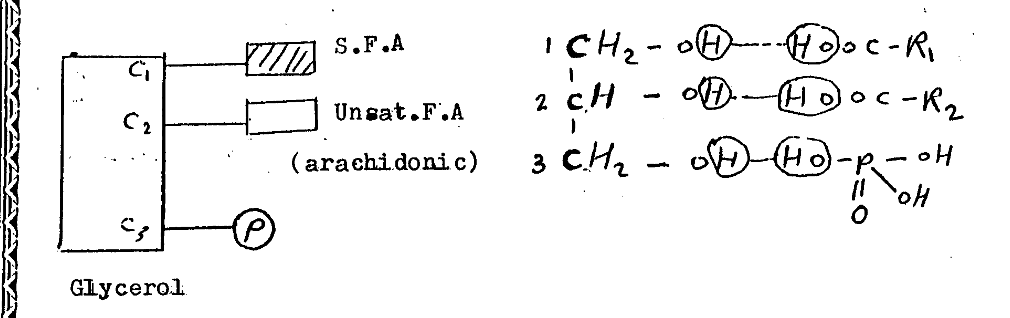
I – phospholipids

Are lipids conjugated with phosphoric acid radicle.

Types:

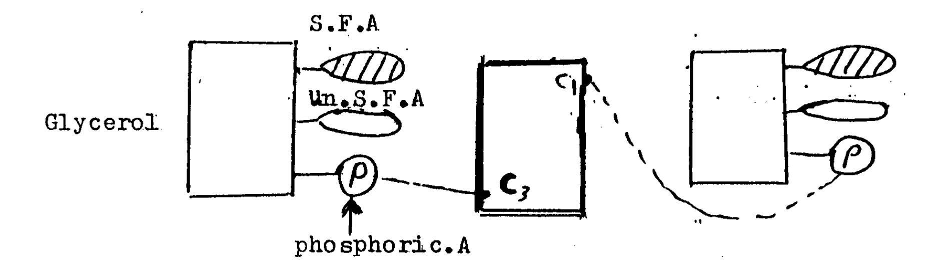
1- Phosphatidic acid:

- Is precursor of other phospholipids.
- Composed of glycerol and two F.A (one saturated, the other unsaturated) and phosphoric acid attached to C_3 of glycerol.



2- Cardiolipin:

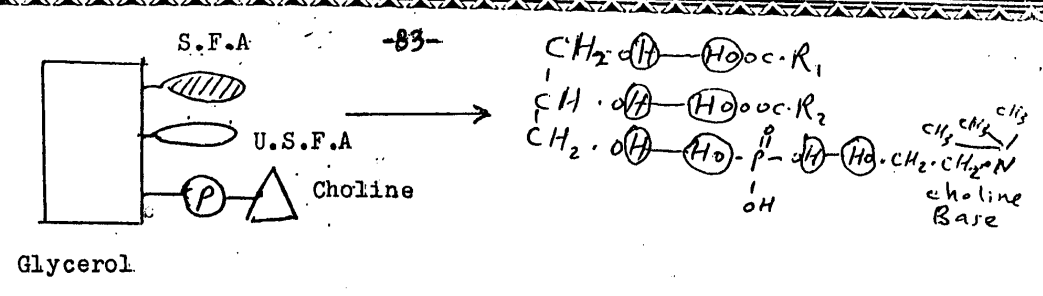
- Composed of two moles of phosphatidic acid, linked together through third mole of glycerol through their phosphoric acid.
- Present in mitochondrial membrane of heart cells.
- Cardiolipin can stimulate antibody formation (antigenic)



3- Phosphatidyl choline [lecithin]:

- Composed of glycerol, one saturated F.A., other unsaturated F.A, H_3PO_4 (as in phosphatidic acid), in addition to choline (nitrogenous base)
- Choline is a strong base, water soluble, member of vit. B complex, enter in formation of phospholipids in liver, its absence → fatty liver.
- Choline is derived from serine $\xrightarrow[-CO_2]{\text{decarboxylation}}$ colamine $\xrightarrow[+[CH_3]_3]{\text{methaylation}}$ choline

$$\underset{\text{Serine}}{\underset{OH\quad NH_2}{CH_2.CH.COOH}} \xrightarrow{-CO_2} \underset{\text{Colamine}}{\underset{OH}{CH_2\text{-}CH_2.NH_2}} \xrightarrow{+3CH_3} \underset{\text{Choline}}{\underset{OH}{CH_2.CH_2.N[CH_3]_3}}$$



LECITHIN

- *Source*:

Cell membrane, egg yolk, and liver, plasma lipoprotien, bile, lung surfactant, and red cells (50% of its membrane is ph.lipid).

- *Importance*:

1- Lecithin is soluble in water and fat solvents, (amphipathic).

2- It acts as lipotropic factor (prevent fatty liver).

3- Lecithin (dipalmityl lecithin) acts as surfactant in the lung that lines the alveoli, it lowers the surface tension that prevent alveolar collapse (adherence).

4- Infants born deficient in lung lecithin (premature babies) suffer rom resp distress syndrome (Hyaline Membrane Disease), and should treated in incubator by surfactants (dipalmityl lecithin).

5- Lecithin with free cholesterol and in presnce of LCAT (lecithin cholesterol acyl transferase), yields cholesterol ester which transported to liver and excreted as bile salts (decrease blood cholesterol and lessen atherosclerosis)

$$\text{Lecithin + free cholesterol} \xrightarrow{\text{LCAT}} \text{cholesterol ester + lysolecithin}$$

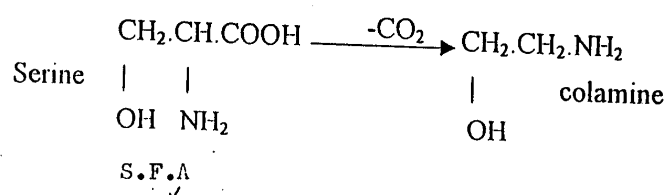
6- Lysolecithin is produced also by action of snake venom lecithinase on RBCs causing hemolysis.

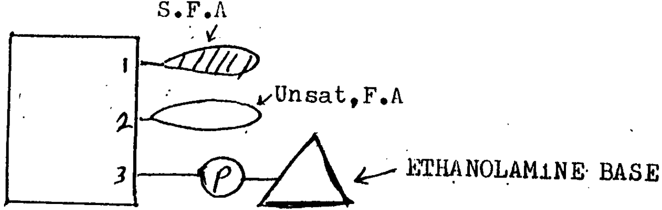
$$\text{Lecithin} \xrightarrow[\text{snake venom}]{\text{lecithinase}} \text{lysolecithin + unsaturated F.A}$$

7- Lecithin in bile solubilizes cholesterol prevent cholest. stones.

4- Cephalin (phosphatidyl ethanolamine):

- *Source*: cell membrane, nerve sheath, and liver.
- *Structure*: similar as lecithins, but the base is ethanolamine [colamine]

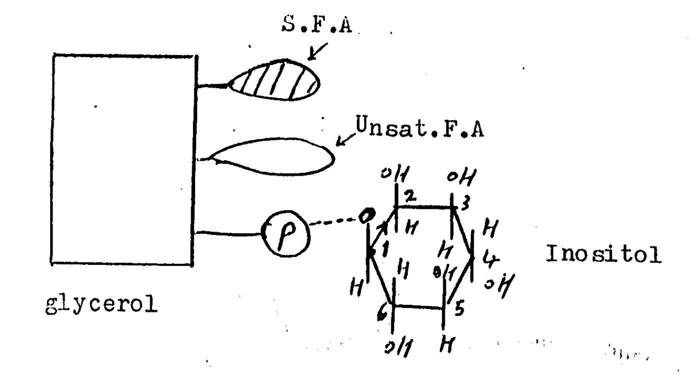




- *Importance*: it accelerates blood coagulation (enter in structure of thrombokinase).

5- Lipositol (phosphatidyl inositol):

- *Source*: cell membranes
- *Structures*: similar to lecithin, but it contains inositol [member of vit. B, lipotropic] instead of choline.



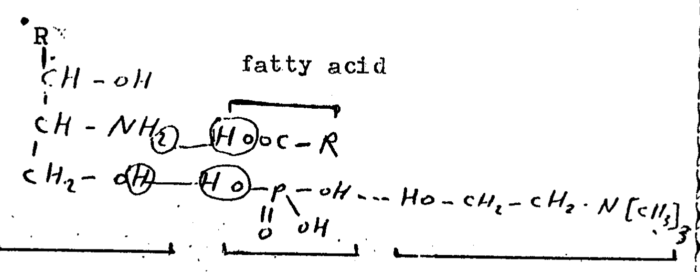
- *Importance*: involved in mechanism of hormonal action (as second messenger)

6- Plasmalogens (phosphatidyl ethanolamine): → C_1 HO.CH=CH.R ... Ⓟ—△ choline or ethanolamine in heart cells

- *Sources*: brain and muscles, also in mitochondria.
- *Structures*: similar to lecithin, but C_1 of glycerol contains unsaturated alcohol [HO-CH=CH-R] instead of fatty acid.
- *Importance*: Plasmalogens are deficient in rare Zellweger syndrome (cerebro.hepato.renal.synd.) (brain damage, also kidney are affected before reaching an early death (and hepatomegaly) due to inability to oxidize long chain unsat.f.A; C26-C39, so accumulate in all tissues.

7- Sphingomyelin:

- *Sources*: brain and nerve tissues
- *Structure*: has no glycerol, but:
  1) Sphingosine base (is amino alcohol)
  2) Fatty acid attached to amine group of sphingosine base.
  3) Phosphate
  4) Choline



*R=$CH_3.(CH_2)_{12}.CH=CH$

- *Importance*: sphingomyelin accumulates in large amounts in liver due to deficiency of sphingomylenase enzyme leading to mental retardation, death in early life [Niemann-Pick's Disease]

- Importance of Phospholipids

1- Form bilipid layer of cell membranes.

2- Lecithin acts as surfactant to prevent collapse of the lung.

3- Cephalin accelerates blood clotting.

4- Lipositol acts as second messenger for hormonal action.

5- Cardiolipins in mitochondrial membranes used for diagnosis of syphilis.

6- Phospholipids are the abundant fat in HDL. (useful type of lipoprotein)

7-Lecithin in bile prevents formation of bile stones.

GLYCOLIPIDS [GLYCOSPHINGOLIPID] CEREBROSIDE

Are lipids containing carbohydrates abundant in nerve tissue and brain.

- *Structure*: sphingosine base + saturated fatty acid [C_{24}] + galactose or glucose.

  [Sphingosine base + F.A. = Ceramide]

- Glycolipids are divided into:

  1) Cerebrosides 2) Gangliosides 3) Ceramide oligo saccharides

1) Cerebrosides

Are divided into: kerasin, nervon and phrenosin [cerebron] according to type of fatty acid.

	Kerasin	Nervon	Cerebron
Sphingosine base	+	+	+
Fatty acid [C_{24}]	Sat.lignoceric.A	Unsat.nervonic.A	Cerebronic hydroxy.F.A
Galactose or glucose	+	+	+

- Accumulation of sphingolipids [cerebrosides] in phagocytic cells due to defect in B. glucocerebrosidase leads to Gaucher's Disease (mental retardation, hepatosplenomegaly, long bone erosion), in this condition kerasin contains glucose instead of galactose.

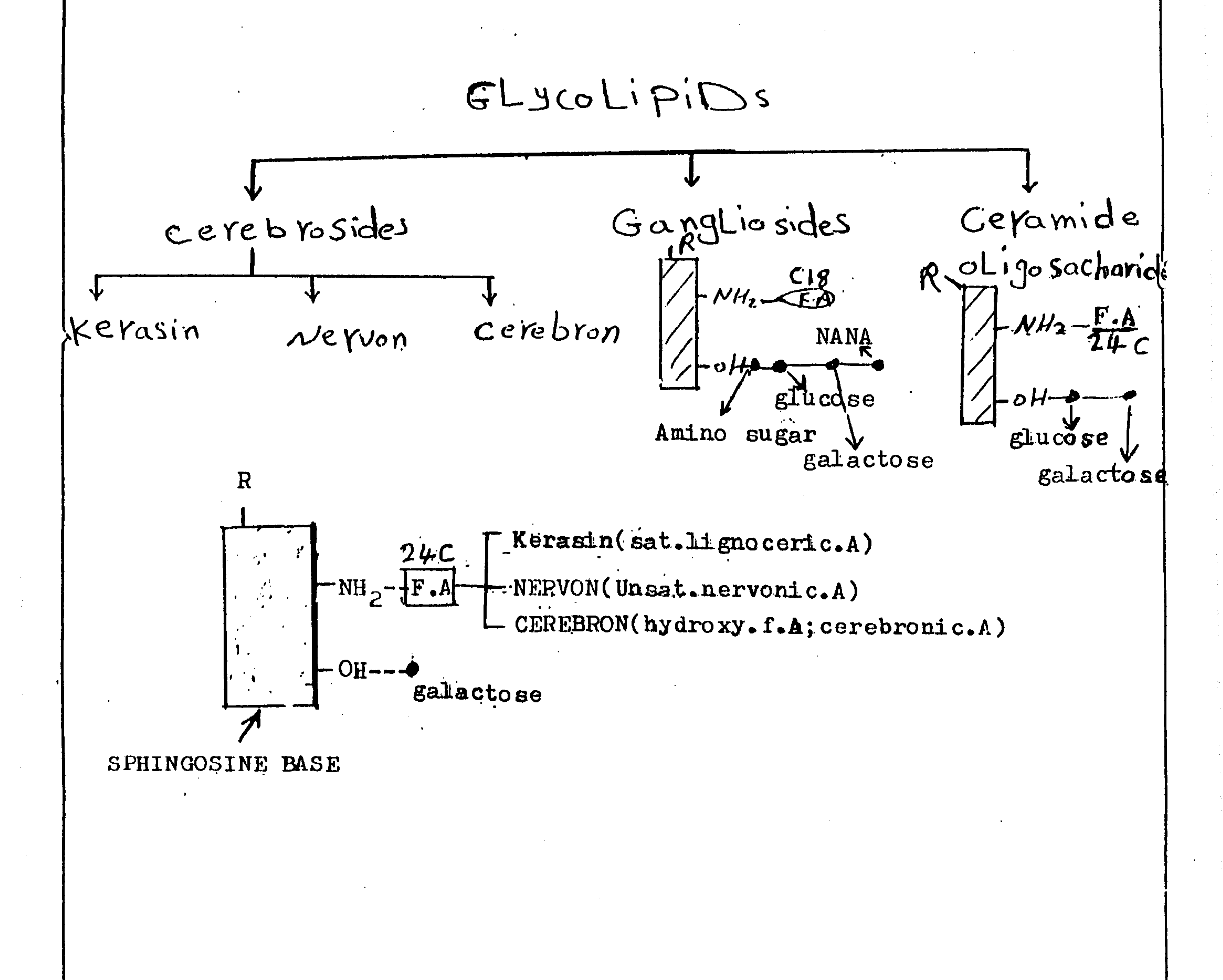
2) Gangliosides

Are present in nervous tissues and spleen.

- *Structure*: sphingosine base, fatty acid, oligosaccaride chain: glucose or galactose, neruaminic acid, sialic acid, and amino sugar.
- *Importance*:
  - Highly concentrated in ganglian cells of C.N.S (5-8% of brain tissues).
  - Act as receptors to toxins of cholera, tetanus.
  - Act as tumour marker on tumour cells.
  - Gangliosides accumulate in brain and viscera due to deficiency of hexosaminidase enzyme which cause blindness, mental retardation, hepatosplenomegaly, and death in early life (Tay-Sachs Disease)

3) Ceramide Oligosaccaride

- *Sources*: heart and kidney
- *Structure*: sphingosine base + fatty acid [C_{24}], many glucose and galactose units.


GLycoLipiDs
cerebrosides
Gangliosides
Ceramide oligosacharide
Kerasin
Nervon
cerebron
R
NH2
C18 F.A
oH
NANA
glucose
Amino sugar
galactose
NH2 - F.A 24 C
oH
glucose
galactose
R
24C
NH2 - F.A
Kerasin(sat.lignoceric.A)
NERVON(Unsat.nervonic.A)
CEREBRON(hydroxy.f.A; cerebronic.A)
OH
galactose
SPHINGOSINE BASE

LIPOPROTEINS

Are compound lipids containing protein, present in:

1) Plasma lipoproteins (soluble).
2) Cell membrane (insoluble).
3) mitochondria (insoluble).

❖ Plasma lipoproteins:

- Lipids are insoluble in the water, therefore must be combined with polar compounds to be soluble in aqueous phase of plasma.
- The polar compounds are proteins (apoprotein), and amphipathic lipids (phospholipids) whose conjugate with (other non polar) lipids, forming lipoproteins which are easily transported in the blood.
- Structure of lipoprotein molecule:
  - Lipid part is inside formed of core of triglycerides and cholesterol ester (very hydrophobic).
  - This lipid core is surrounded by another core of amphipathic lipids (phospholipids, free cholesterol)
  - Protein core (apoprotein) surrounds the amphipathic lipid layer, forming water soluble lipoprotein, which is absorbed from intestine and transported in the blood as chylomicrons.

•Plasma lipoprotiens act as transporter of lipids in plasma to tissuses for oxidation.

❖ Separation and types of plasma lipoproteins:

Can be carried out by:

1) Electrophoresis
2) Ultracentrifugation
3) Gas liquid chromatography
4) Thin layer chromatography

1) Electrophoresis:

Lipoproteins migrate in an electric field depending upon their charges in the buffer media, and the molecular weights.

So, α-lipoprotein migrates faster than β-lipoproteins.

- Classes:

1- **Chylomicrons**:

Don't migrate (big, high m.w. mole), contain mainly triglycerides absorbed from intestine [99%-lipids, 1% protein].

2- β **- lipoproteins**:

Transport about 50-60% of plasma cholesterol to all body cells (80% lipids, 20% protein; apoprotein B_{100})

3- **Pre'β- lipoproteins**:

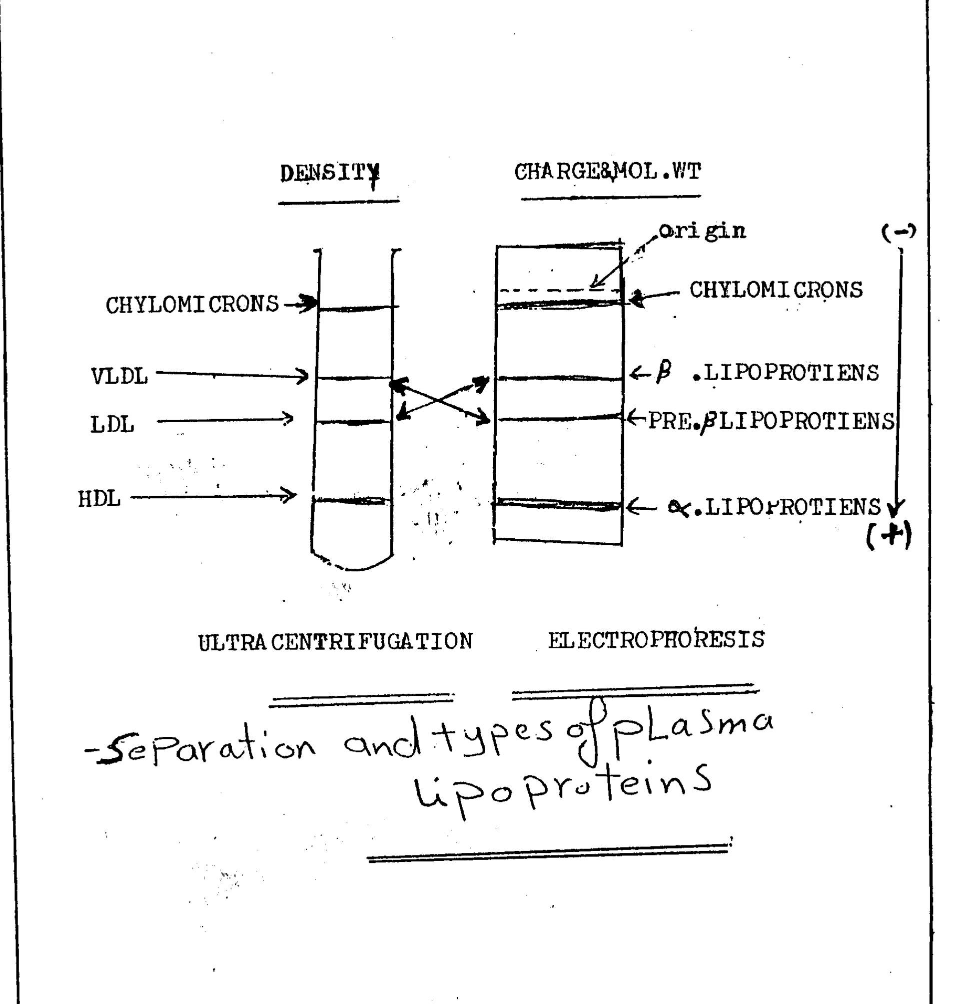
Transport T.G formed in liver to peripheral and adipose tissues (90% lipids, 10% protein).

4- α- **lipoproteins**:

Remove cholesterol from peripheral tissues to liver (total lipids 55%, and 45% proteins, Apo A), has the highest percentage of phospholipids 30-40%.

2) Ultracentrifugation:

Is separation of plasma lipoproteins with very high speed centrifugation into layers according to their density, as protein content increases, the density will be increased.



-Separation and types of plasma lipoproteins

Classes, composition, and function of plasma lipoproteins

Classes Ultracentrifugation (Density)	Total proteins % (Apolipoproteins)	Total lipids	T.G.	Cholest Ester	Cholest	Ph. Lipid	Size of molecule (nm)	Source	Function
Chylomicrones	(1%): B-48, C, E, A	99 %	90	3	1	6	150-1000	Diet	Transfer lipid diet to liver adipose tiss. and peripheral tissues
VLDL (pre β lipoprotein)	(10%): B-100, C	90%	60	14	6	20	30-70	Liver	Transfer T.G from liver to tissues, give rise to LDL
LDL (β lipoprotein)	(20%): Apo, B100	80%	18	45	10	27	17-26	VLDL	Major carrier of cholesterol from liver to peripheral tissues
HDL (α lipoprotein)	(45%): •Apo-A, C, E in liver In plasma: •Apo-A_1 (75%) A_2 (25%)	55%	15	30	10	45	7-9	Liver	•Remove cholest. From peripheral tiss. to liver. • Stim. LCAT

* Apo A_1 : Activator of LCAT   * Apo C_{11} : Activator of lipoprotein lipase in chylomicrons

:* Apo B, E : Recognition of lipoproteins by its cell receptors

- Classes:

1- **Chylomicrons**:

Contain the lowest protein, and the highest lipids, so float on surface (99% lipids, 1% protein)

2- **VLDL**:

Very low dense lipoprotein (90% lipid, 10% protein), and is alternative to pre β-lipoprotein, its layer is below the chylomicrons.

3- **LDL**:

Low dense lipoprotein (80% lipid, 20% protein), is alternative to β-lipoprotein, its layer is below VLDL.

4- **HDL**:

High dense lipoprotein (55% lipids, 45% protein), is alternative to α-lipoprotein, is the lowest layer (highest in protein; high dense)

DERIVED LIPIDS
STEROLS & STEROIDS

They are produced by hydrolysis of simple or conjugated lipids, they include the following:-

1- Fatty acids,

2-Alcohols(has OH group): as cholesterol, Vit.D

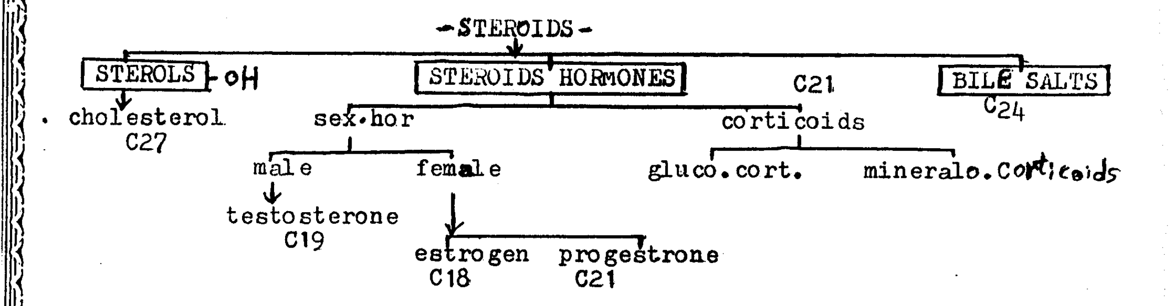
3- Steroids :
Are cyclic compounds containing cyclopentano-perhydro-phenanthrene ring, has 17 C ,OH group at C_3 as :sex.Hor&corticoids, and bile acids.

4-Carotenoids:
As carotenes, lycopene, and carotenols.

5- Fat soluble vitamins : Vit; A,D,E,K .

6- Some carcinogenic substances as methyl cholanthrene.

* GENERAL STRUCTURE OF STEROIDS:
  - Consist of four rings (A, B, C, and D)
  - Have (-OH) or (=O) groups at C_3.
  - Have $-CH_3$ group at C_{10}, C_{13}.
  - Have side chain(R) at C_{17}.
  - (A&B rings contain double bond, but C&D rings are always saturated.



CHOLESTEROL

❖ Structure:

- Contain (-OH) at C_3 (so it is considered as alcohol)
- Contain 2 methyl groups at C_{10}, C_{13}
- Has double bond between C_5, C_6
- Has long side chain at C_{17}
- Is cyclic compound containing 27 C atoms ($C_{27}H_{45}.OH$)

-CHOLESTEROL-

❖ Occurrence:

- Present in cell membrane of every cell, liver kidney, brain, nerve tissues, and egg yolk, chicken skin, bile(390%).

- Cholesterol is very hydrophobic, so must be associated with proteins (lipoproteins) to be transported in the blood, **mainly as LDL.**

❖ Blood level:

- 150-250mg %, (2/3 blood cholesterol is synthesized in liver, the remain 1/3 exogenous from diet)
- 30% is free cholesterol, 70% is in ester form (very hydrophobic) conjugated with fatty acid at C3 (linoleic; 50%, or oleic; 18%, or palmitic; 11%, and arachidonic 5%).

❖ Importance:

1) Enter in structure of every body cell **membrane.**
2) It is the precursor of all sex, cortical hormones.
3) Oxidized in liver to give cholic acid which forms bile salts.
4) Cholesterol in liver is oxidized $\xrightarrow{-2H}$ 7 dehydrocholesterol which give rise to vit. D_3 under skin by U.V rays, through opening of ring B

U.V RAYS

7.Dehydrocholesterol

Cholecalciferol (Vit.D_3)

Bile Acids & Bile Salts

- Primary cholic and chenodeoxy cholic acids are synthetized in liver from cholesterol by hydroxylation, oxidation, and reduction reactions,

- They have carboxyl group at C_{24} and (-OH) group [at 3,7,12 in cholic A, at 3,7 in cheno deoxy cholic A)....primary cholic acids.
- They conjugate in liver with glycine or taurine to form glycocholic A, or taurocholic acid, and excreted in bile as their sodium or potassium salts (bile salts)
- Secondary bile acids: (deoxycholic and lithocholic) are derived from primary acids after removal of the amino acids (glycine and taurine), also (-OH) from C_7 is removed, by intestinal bacteria, secondary acids are formed (deoxy cholic acid is partly reabsorbed to liver, and lithocholic acid; not reabsorbed).
- Secondary bile acids are reduced in intestine to coprosterol which is excreted with stool. giving its colour.

$H_2N.CH_2.COO.Na$
sod. glycocholate

(BILE SALT)
.Has amino acid

PRIMARY CHOLIC ACID
(-OH at 3,7,12)

SECONDARY deoxy.cholic acid
(No -OH at C_7)

❖ **Importance**:

1- Bile salts are amphipathic (has polar –OH groups), so emulsify lipids in intestine to render them easily attacked by pancreatic lipases.
   - When fat digestion is impaired, other food stuffs are also impaired because the fat covering the food particles prevents other enzymes from attacking them.

2- Bile salts are significant route for cholesterol excretion from the body through the bile, because body tissues can't breakdown the large steroid nucleus of cholesterol.

❖ Ergosterol:

- Is plant sterol, occurs in ergot and yeast, is poorly absorbed from small intestine, gives vit. D_2 by U.V rays.

Steroid Hormones

A) Steroidal sex hormones

B) Steroidal corticoal hormones

A- Steroidal sex hormones (male and female sex hormones); ARE CYCLIC COMPOUNDS DERIVED FROM CHOLESTEROL.

1) Female sex hormones:

a- Estrogens

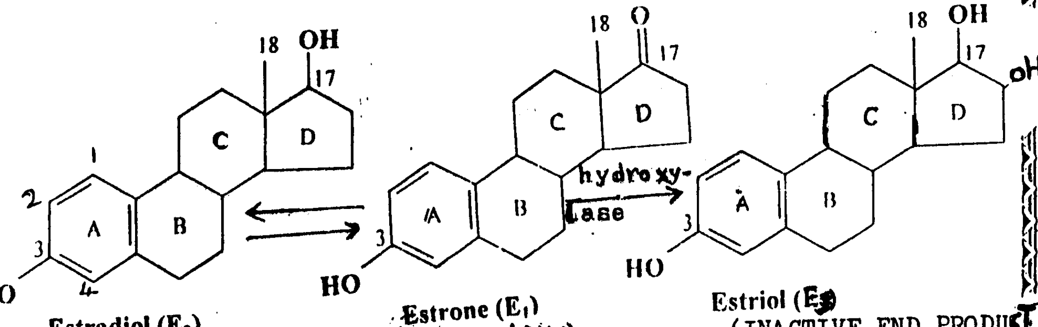
Site:

Estrogens are produced from cholesterol:

1) Ovary and placenta in female (ovary contains 100,000-200,000 ova, used only 400-500 in life.).
2) Adrenal cortex in male and female
3) Testis in male to lesser extent, but if increased---→ gynecomastia at pubirty, and in older ones if there is chronic liver disease.

Types:

1) Estradiol [E_2]: the most active estrogens
2) Estrone [E_1]: less active, produced in liver from metabolism of E_2.
3) Estriol [E_3]: the least active produced by metabolism of E_1 and E_2 in liver.



- It has [–OH] at C_3, and [–OH or =O] at C_{17}, has no CH_3 at C_{10}, but CH_3 at C_{13}, its carbons [C_{18}].

Cholesterol → androstenedion → testosterone $\xrightarrow[-CH_3]{aromatase}$ estradiol (E_2) → E_1 → estriol (E_3)

Function:

1) Stimulate secondary sex female characters
2) Development of female sex organs
3) Estradiol has anabolic effects on bone & cartilages, so at menopausal age, females may suffer from osteoporosis.
4) Synthetic estrogens [ethinyl estradiol] used as oral contraceptive in combination with small amounts of progestrone.

Fate:

Are inactivated in liver to estriol then conjugated with glucouronic acid which excreted in urine.

-Estriol(E_3)is produced in largest amounts at late of pregnancy, it reflects fetoplacental function;if decreased-->pregnancy hazards.

b- Progestrone

Site:

1) **Ovary**: (by corpus leuteum in second half of menstrual cycle, and during first 2 months of pregnancy) ; to fixes pregnancy.
2) **Placenta**: during mid and late period of pregnancy (its production is 30-40 times than corpus leuteum in late of pregnancy)
3) **Adrenal cortex**: in both males and females

Structure:

Ketone group [=O] at C_3, double bond between C_4, C_5, and CH_3 groups at C_{10} and C_{13}, methyl ketol at C_{17} (-CO.CH_3)

Function:

- prepares uterus for implantation of ovum
- prevents abortion
- stimulates breast acini during puberty and pregnancy
- inhibits milk production in late pregnancy, then decrease abruptly after delivery causing lactation.

- antagonizes the action of estrogens in various tissues.
- Acts as indicator of ovulation; (↓ peripheral blood flow → ↓ heat loss → ↑ body temp. by 0.5°C during luteal phase of the cycle),

Fate:

In liver is inactivated to pregnanediol which is conjugated with glucouronic acid and excreted in urine, and bile.

Cholesterol → active progestrone --liver--> inactive pregnanediol

2) Male sex hormones [Androgens]

Sites:

1) Leyding cells in testes from cholesterol.
2) Adrenal cortex in males and females

Types:

1) Testosterone: the main hormone [double bond at C_4 and C_5]; 95% .
2) Dihydrotestosterone (DHT): derived from testosterone, at the target cells, is the active form of testosterone; 2-4%.
3) Dehydroepiandrosterone and androstenedione: are the precursors of testosterone, less active.

Cholesterol --testes--> pregnenolone --testes--> dehydroepiandrosterone [DHE] --testes--> androstenedione --testes--> testosterone --tissues--> dihydrotestosterone (DHT)

Testosterone --Reductase / NADPH--> DHT Dihydrotestosterone "THE ACTIVE FORM"

Structure:

- Ketone group [=O] at C_3, double bond between C_4 and C_5, $-CH_3$ groups at C_{10} and C_{13}, [-OH] at C_{17}.

Function:

1) Stimulate growth of male sex organs, and secondary sex male characters (voice, hair,...)
2) Stimulate spermatogenesis
3) Anabolic effect on proteins. ,IMPROVE MUSCLE PERFORMANCE, SO TAKEN BY SPORTSMEN.

Fate:

- Androgens arised in testis, renal cortex are inactivated in liver (by oxidation of ring D and reduction of ring A), then conjugated with glucouronic acid and excreted in urine as 17-ketosteroids.
- 17-ketosteroids: has ketone group [=O] at C_{17} instead of [-OH]
- This group includes dehydro epiandrosterone acetate [DHEA], androsterone, epiandrosterone.
- Normal values of 17-ketosteroid: 5-15 mg/day in females and 10-20mg/day in males.
- This value increases in hyper adrenocortical function [Cushing's Syndrome], and in testicular tumours.
- Decreased levels in hypoadrenocortical function (Addison's Disease) and in male hypogonadism.

B- Steroidal cortical hormones

Are derived from cholesterol in adrenal cortex

Structure:

- All have ketone group [=O] at C_3
- All have double bond between C_4 and C_5
- All have 2 methyl groups at C_{10}, C_{13} (except aldosterone)
- All have ketol group [-CO.CH_2OH] at C_{17}
- Glucocorticoids have [-OH] or [O] at C_{11}
- Its carbon atoms C_{21}

Types:

I) Glucocorticoids

II) mineralocorticoids

I) Glucocorticoids

Structure:

Ketone group [=O] at C_3, double bond $C_4 \rightarrow C_5$, ketol group at C_{17}

Types:

1- Cortisol (Hydrocortisone):

Is 17-hydroxycorticosteron, (-OH) at C_{17} ketol group, is the most potent corticosteroid.

2- Cortizone (11-dehydro 17-hydroxy corticosterone)

3- 11-dehydrocorticosterone

Function:

- Glucocorticoids have anabolic effects on carbohydrates (increase blood sugar by stimulation of gluconeogenesis, decrease glucose oxidation in body, has anti-insulin effect, inhibit hexokinase)
- Has catabolic effects on fat and proteins (increase fat metabolism, increase ketone bodies.
- Decrease immune response in acute allergy.

II) Mineralocorticoids

Arised from cholesterol in adrenal cortex

Types:

1- Aldosterone:

- Structure as corticosterone but has [-CHO] instead of [$-CH_3$] at C_{13}
- Is the most potent mineralocorticoids, has some glucocorticoid action (has –OH at C_{11})

2- 11-deoxycorticosterone (DOC):

- Is less potent, has no glucocorticoids action.
- Its synthetic derivatives is deoxycorticosterone acetate [DOCA], used in treatment of Addison's Disease [Cortical Hypofunction]

Function:

Mineralocorticoids promote renal reabsorbtion of Na^+, Cl^-, and HCO_3^- to blood, so keep blood pressure, and promote excretion of K^+, Mg^{++} to urine

Fate:

Mineralocorticoids are inactivated in liver by reduction of ring A, then conjugation with glucouronic acid to be excreted in urine and bile.

Carotenoids

Are derived lipids, has yellow to red pigment, are provitamin A

Types:

I) **Carotenes**:

- Are hydrocarbones only [C and H]
- It includes:
  - α, β, γ carotenes, has yellow pigment, present in human fat, butter, carrots, apricots...etc.
  - lycopene: is red pigment present in tomatoes, and water melon ,it has anti oxidant action.

II) **Carotenols**:

- As carotenes, but are oxygenated hydrocarbones [C, H and O], are present in egg yolk and oranges.

Importance:

- They are converted to vit. A in intestine.
- Carotenoids reduce the risk of neoplast changes in lung, oral cavity and urinary bladder due to its strong antioxidant property by scavenging of oxygen free radicles which damage DNA.

CH_2OH 21
C=O 20
18, 17, 19, 11, 3, O

11-deoxycorticosterone (DOC)

CH_2OH 21
C=O 20
HO 18, 17, 19, 11, 3, O

Cortisol

-OH at C11

+OH at C17

CH_2OH 21
C=O 20
HO 18, 17, 19, 11, 3, O

Corticosterone

-H at C11

CH_2OH 21
C=O 20
O 18, 17, 19, 11, 3, O

+ OH at C17

+CHO at C13

-H at C11
+OH at C17

CH_2OH 21
C=O 20
18, O=CH, HO, 17, 19, 11, 13, 1, 3, O

Aldosterone

CH_2OH 21
C=O 20
OH
18, O, 17, 19, 11, 3, O

Cortisone

mineralocorticoids).

Glucocorticoids:

ADRENAL CORTICAL HORMONES
(CORTICOIDS)

Cholanthrenes

- Are lipids soluble compounds, related in structure to steroids, but with an extra methyl ring, its rings are unsaturated.
- Methyl colanthrene is one of the most active carcinogenic substance as polycyclic hydrocarbones.
- If they gained to body, are stored in depot fat, and affects nucleus causing disordered cell division.

CH_3

* METHYL CHOLANTHRENE*

NUCLEOPROTEINS AND NUCLEIC ACIDS

Definition:

Nucleoproteins are present as conjugated proteins; protein part as basic simple protein, which is attached to a non protein part (nucleic acid).

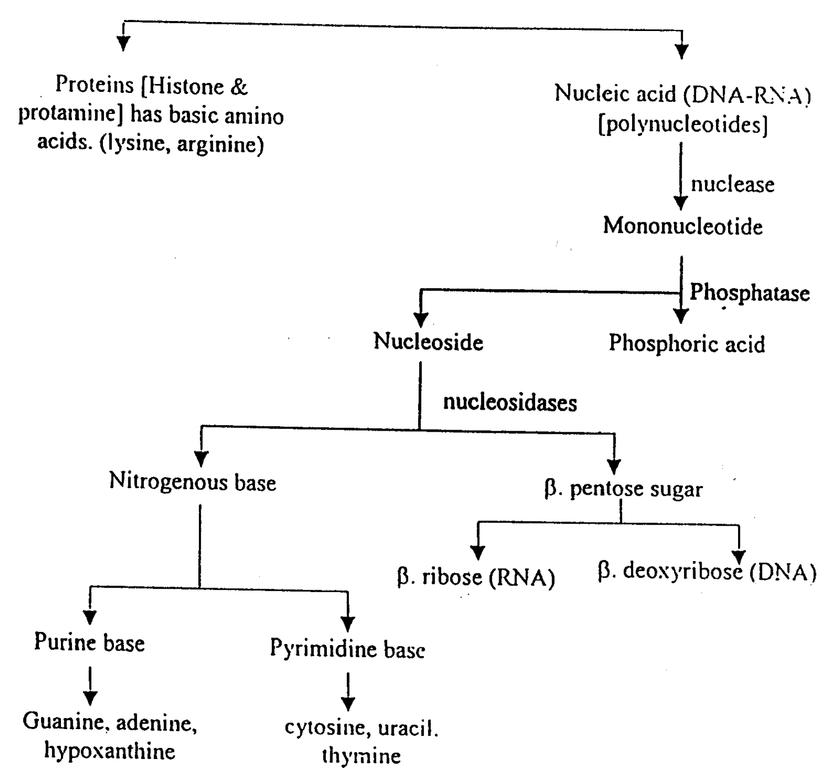
Occurrence and Function:

Nucleoproteins are present in all mammalian, plant cells, bacteria and virus.

DNA: Is present in nucleus, it forms the chromosomes which takes part in cell division, and carry the hereditary genes.

RNA: is present in cytoplasm and associated with protein synthesis in ribosome.

Structure: By: hydrolysis:



So,

Mononucleotide = one base + pentose sugar + one or more ph. group.

Nucleoside = one base + pentose sugar; (No ph. group).

Bases = Purine or pyrimidine:

URACIL — RNA UNIT

THYMINE — DNA UNIT

Pyrimidine

HO[2] / NH_2 [4] → Cytosine (lactim)

HO [2,4] → Uracil

Uracil — CH_3 [5] → Thymine (lactim)

Purine

H_2N [6] → Adenine (lactim)

HO [6] → Hypoxanthine (6-oxypurine)

Hypoxanthine — H_2N [2] → Guanine (lactim)

Fate of end product bases:

All types of bases are reused in body for RNA and DNA synthesis; except hypoxanthine which is a waste product:

$$\text{Hypoxanthine} \xrightarrow[\text{FAD + (Molybdenum)}]{[O]\ \text{X. oxidase}} \text{xanthine} \xrightarrow[\text{FAD}]{[O]\ \text{X. oxidase}} \text{uric acid}$$

So, uric acid is the end product of purine base in body, if increases → gout, kidney stones.

** Uric acid (less soluble) in plasma is present as mono-Na. urate (97%) is more soluble , relatively insol. at normal blood pH, but if level ↑ above 6.4mg% is saturated and form urate crystals which may PPT. in tissues and joints (gouty arthritis).

** Plasma urates is considered now as potent antioxidant inspite of being a waste product, also plasma vit. C and bilirubin are considered as antioxidants in addition to urates.

Biochemical Importance of Naturally Occurring Free Nucleotides:

A) Purine Derivatives:

i) Adenosine derivatives:

1. ATP:

Is energy donor, has high energy phosphate.

2. ADP and AMP:

Are phosphorylated to yield high energy ATP

Adenine

Ribose

Adenosine 5'-monophosphate (AMP)

Adenosine 5'-diphosphate (ADP)

Adenosine 5'-triphosphate (ATP)

3. cAMP:

Is arised from ATP by adenyl cyclase, and inhibited by ph. diesterase.

Is major regulator of cellular metabolism, secondary messenger for many hormones.

ATP → (ADENYLATE CYCLASE, PP_i) → CYCLIC 3',5'-AMP → (PHOSPHODIESTERASE, H_2O) → AMP

4. Active sulphate: [Sulphate + nucleotide].

As ph. adenosine phosphate phosphosulphate (PAPS).

AMP ~ P ~ P (ATP)

SO_4^{2-} + ATP (Mg^{2+}) → P~P + ADP

ADENINE —RIBOSE —Ⓟ—O—SO_3^{2-} (with Ⓟ on ribose)

"ACTIVE" SULFATE (ADENOSINE 3'-PHOSPHATE-5'-PHOSPHOSULFATE)

The formation of adenosine 3'-phosphate-5'-phosphosulfate.

Is sulphate donor in synthesis of proteoglycans and conjugation of Xenobiotics (as phenols, alcohols).

5. *Active methionine:* [methionine + nucleoside].

As s. adenosyl methionine, is methyl donor for methylation reactions.

$$\underbrace{A.R}_{} \sim \overset{|}{s}.CH_3 \quad CH_2.CH_2.\underset{NH_2}{CH}.COOH =$$

Nucleoside
(adenosine)

The structure of S-adenosylmethionine

ii) Guanosine derivatives:

1. *GTP:*

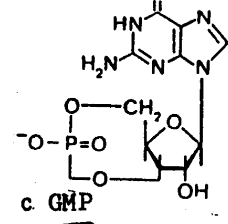
Is high energy phosphate for some cell reactions.

2. *GDP:*

Is phosphorylated to GTP in citric acid cycle

- Succinyl-CO.A+Pi+GDP $\xrightarrow[\text{thiokinase}]{\text{succinate}}$ Succinate+GTP+CO-A.SH

GTP and G. proteins are important in catalytic activity of adenyl cyclase, energy source for protein biosynthesis.



c. GMP

3. *cGMP:*

Is similar to cAMP, acts as regulator of some intracellular reactions.

iii) Hypoxanthine derivatives:

As inosinic acid (IMP) inosine monophosphate; is hypoxanthine-ribonucleotide [hypoxanthine + ribose + Ph]. Is precursor of all adenosine nucleotides.

B) Pyrimidine Derivatives:

1. Uridine diphosphate-sugars:

Are precursors of glycogen and proteoglycans synthesis.

2. UDP-glucouronic acid:

For synthesis of proteoglycans, conjugation reactions in the body.

3. CDP-choline:

For synthesis of some phospho. glycerides, and sphingomyelins.

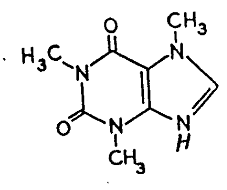
C) Vitamin Nucleotides:

1. **NAD and NADP:** Containing niacin as hydrogen carrier.
2. **FAD and FMN:** Containing riboflavin (B_2) as hydrogen carrier.
3. CO-A: Containing pantothenic acid, as acid carrier.
4. Vit B_{12}: Acts in body after being converted to deoxynucleotide.

Plant Xanthines:

- Free bases in cells include hypoxanthine and xanthine as intermediate metabolites of adenine and guanine, and their end product is oxidized to uric acid, excreted in urine.
- Plants contain methylated xanthines which include:

1. **Caffeine** (1, 3, 7 trimethyl xanthine) of coffee.
2. **Theophylline** (1, 3 dimethyl xanthine) of tea.



Caffeine (1,3,7-trimethyl-xanthine)

Theophylline (1,3-dimethyl-xanthine)

Theobromine (3,7-dimethyl-xanthine)

3. **Theobromine** (3,7 dimethyl xanthine) of cocoa.

   * Each of tea, coffee, coca contain all types of the methyl xanthines, but mainly the above described type.

- Methyl xanthines are absorbed and partly demethylated in liver to free xanthine → uric acid.

  The remain is not demethyated (doesn't change to uric acid).

  * Cup of coffee = 100-150 mg caffeine
  * Cup of tea = 25-30 mg caffeine.

	Caffeine (coffee) $3CH_3$	Thiophylline (tea) $2CH_3$	Theobromine (coca) $2CH_3$
Absorption	+	±	+
S. Stimulant action on C.N.S and meddula	+++	++	±
Diuretic action	+	++++	++
Stomach irritation (nausia)	- (but ↑ H Cl)	++	-

Synthetic Analogs of [Bases, Nucleosides, or Nucleotides]:

- Nucleic acids are important for cell division and growth.
- Nucleotide is the monomers of the nucleic acids.
- To inhibit cell division and growth, a synthetic nucleotide or base similar to the natural one is used to compete inhibition for cellular enzymes responsible for cell growth and division.
- These synthetic nucleotides analogs are used for treatment of cancer, viral (DNA or RNA) infections, and to inhibit rejection of the transplanted organ by inhibition of the immune response.

1. Anticancer uses:

5-Fluorouracil

As 5-fluoro or 5-iodo derivatives of uracil (base) or deoxy uridine (necleatide) as:

6-mercapto purines, 6-Aza uridine, 6-Aza-guanine.

6-Mercaptopurine

2. Antiviral:

For treatment of viral hepatitis, AIDS:

5-iodo deoxy uridine, arabinosyl cytosine, arabinosyladenine.

Anticancer drugs can be used as antiviral.

3. In organ transplantation:

As Azathioprine (AZT).

Azathioprine

4. Allopurinol:

Is purine analog for inhibition of xanthine oxidase to (inhibit catabolism of purines to uric acid, as in cases of gout.

Allopurinol (lactim)

URIC ACID

NUCLEIC ACIDS

Are polynucleotides, subdivided into RNA and DNA (each nucleotide formed of base, pentose and ph).

I. RNA:

Is polynucleotide chain present as one strand:

Bases: Has two purine bases (guanidine and adenine] and two pyrimidine bases [cytosine and uracil], present as single stranded molecule

Sugar: β. ribose [in which C_1 of ribose is attached to the base, (C_1 in pyrimidin, C_9 in purine.) and C_5 of ribose in attached to the phosphoric acid].

Classes and function: All are formed in nucleus under control of DNA and RNA polymerasc enzyme, but act in cytoplasm.

1. Messenger RNA (mRNA):

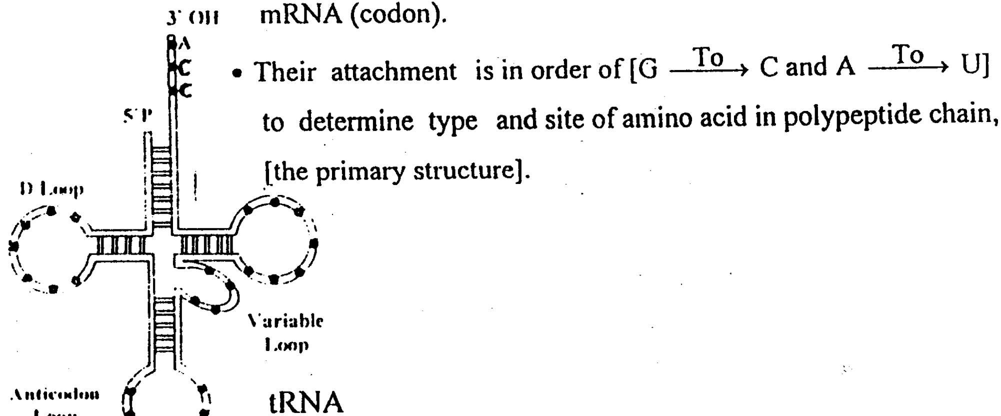
- Is single stranded polynucleotides, formed in nucleus under control of DNA (acts as template for protein biosynthesis), its nucleotides ranged from 400-4000.
- Every 3 bases are arranged to form the codon, which transcripts the genetic code of the DNA to the desired protein synthesis (transcription).
- Every 3 bases on nucleotides are required for one amino acid.

AUG GUA GCA

mRNA codon

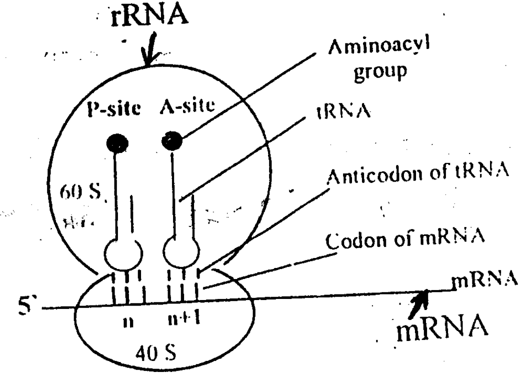
2. Transfer RNA (tRNA):

- Its nucleotide chain is arranged to form 3 loops and two free ends, where amino acids are attached to it.
- It consists of about 75 nucleotides.
- It acts as carrier of amino acids to the site of protein synthesis in ribosome.
- There are specific twenty types of tRNA; one to each amino acid, for protien biosynthesis.
- Its anticodon loop has three bases complement to that bases of mRNA (codon).
- Their attachment is in order of [G $\xrightarrow{\text{To}}$ C and A $\xrightarrow{\text{To}}$ U] to determine type and site of amino acid in polypeptide chain, [the primary structure].



3. Ribosomal RNA (rRNA): Site of protein synthesis:

Formed in nucleus, and pass to cytoplasmic ribosome, has two subunits, the large one is attached to tRNA, and the small one to mRNA.



- By interaction of mRNA and tRNA the process of protein synthesis starts.

•Its nucleotides ranged from 120 -4000 units.

- • HnRNA: Heterogenous nuclear RNA: One strand, 5000-50.000 nucleotides, structure as mRNA, take part in synthesis of mRNA.

II. DNA:

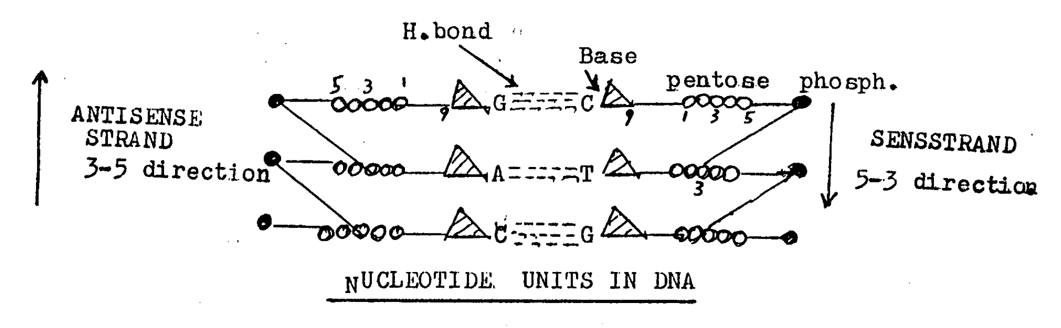
Is neuclic acid formed of long double chain neucleotides, present in nucli.

* Each nucleotide consists of:

1- **Sugar:** β. deoxyribose.

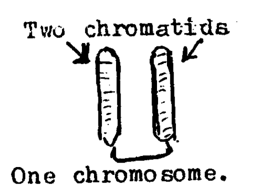
2- **Bases:** It has two purine bases: G & A, and two pyrimidine bases C and T in equal amounts.

- Guanine is attached only to cytosine by 3 H bonds.
- Adenine is attached only to thymine by 2 H bonds; (pairing rule).



NUCLEOTIDE UNITS IN DNA

- The DNA molecule consists of two long nucleotides chain, turned around each other to form double helix (spiral) forming the chromatid.



- Each two similar chromatid form one chromosome, which is fixed in number in any species, in human nucli is 46 chromosomes, but in gonad is 23.
- the total genetic content of the cell is called genome.

3- **Phosphoric acid:** is attached to C_5 of ribose in first nucleotide, and to C_3 of ribose in second nucleotide. ⟶ sense strand.

Function:

1. Replication of DNA (synthesis) and cell devision; (mother cell → 2 similar daughter cells).
2. Transcription: synthesis of mRNA for protein biosynthesis.
3. Store the genetic information of the cell on its genes.

Replication

DNA

Transcription

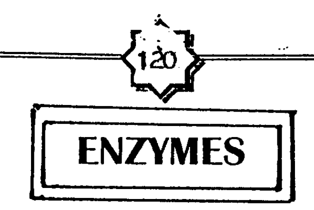
m RNA

Translation

Protien.

The Main Differences Between DNA and RNA:

DNA long chain (million)	RNA (thousand)
1. Occurs in nucli, forms the chromosomes for genetic information; (replication of DNA by cell division), transcription of mRNA.	1. Acts in cytoplasm, involved in protein synthesis by translation.
2. Its sugar is β. deoxyribose.	2. Its sugar is β. ribose.
3. Is double stranded molecule, containing two purine bases (G & A) and two pyrimidine bases (C & T). * The double strand: [G $\xrightarrow{To}$ C and A $\xrightarrow{To}$ T (pairing rule)].	3. Is single strand molecule, containing two purines (G & A) and two pyrimidines (C & U). (codon) (anticodon) mRNA to tRNA order: G $\xrightarrow{To}$ C and A $\xrightarrow{To}$ U].
4. They form double stranded helices.	4. RNA is divided into three classes: i) mRNA, ii) tRNA and iii) rRNA.
5. Synthesis: by replication.	5. Synthesis: by transcription.



ENZYMES

Definition:

Are defined as protein thermolabile catalyst; accelerate the speed of chemical reaction without being consumed in that reaction.

Enzymes are produced by living cells, and can act away from them.

Examples:

- Sucrose can be hydrolysed into (glucose + fructose) in very long time, but in presence of sucrase enzyme, can accelerate that reaction in few hours only.
- Also enzymes can function inside as well as out side the cell; yeast cell contains zymases which can ferment glucose into ethyl alcohol + CO_2 inside the cell, when yeast is crushed, its enzymes also can ferment glucose out side the cell.
- Hormones differ (can act inside the cell only).

Distribution:

I. Intracellular enzyme:

Produced and act inside the cell as metabolic enzymes which breakdown the food stuffs to obtain energy in mitochondria of the cell.

II. Extracellular enzyme:

Produced inside the cell, but act outside the cell, as digestive enzymes, pepsin and amylase.

Importance:

- Enzymes are essential for all chemical reactions required for continuation of life, and gain energy rapidly for that reactions.
- Also cathepsins enzymes are intracellular enzymes for cell autolysis, active only in acid pH due to accumulation of lactic acid in death, but in living body pH is not suitable to that enzymes.

Chemical structure of enzymes:

- Enzymes are formed of thermolabile protein, act as carrier of the enzyme system.
- This protein (polypeptide chain), is in form of primary structure, secondary structure (folded chain), tertiary structure (coiled chain), or quaternary structure (contains more than one chain).
- Enzymes, may be simple proteins or conjugated (Holoenzyme).
- Holoenzyme; consists of protein part, which is attached loosely to non protein part named coenzyme which is the active part of the whole enzyme, is heat stable, or firmly attached to non protein part called the prosthetic group (the active part of the whole enzyme), as catalase enzyme.

LDH NAD Apo enzym prosthetic part catalase

HOLOANZYME

CO-ENZYMES

Definition:

- Are active, non protein, heat stable substances, which are necessary for the activity of the most enzyme system, and without their presence no reaction can take place.
- Vitamin B complex enter in structure of many coenzymes.
- Other hydrolytic enzymes need no coenzymes as lipases and CARBOHYDRASES.

Types:

I. Hydrogen carriers:

Act as hydrogen carriers in oxidoreduction processes.

Classes:

1. NAD^+ and $NADP^+$ (has nicotinamide).
2. FAD and FMN (has riboflavin).
3. Lipoic acid (vit. B member).
4. Coenzyme Q (relation to vit. K).

1. NAD: (Nicotinamide adenine dinucleotide) = CO-I.

- Act as hydrogen carrier in reactions included:

$$[\text{lactate} \xrightarrow[\text{LDH}]{\text{NAD}} \text{pyruvate}].$$

2. NADP: (Nicotinamide adenine dinucleotide phosphate) CO-II:

- Act as hydrogen carrier in hexose monophosphate shunt.

Structure: as NAD, but has extraphosphoric acid at right ribose.

3. FAD: (Flavin adenine dinucleotides):

Act as hydrogen carrier in: (oxidoreduction).

- $NADH + H^+ + FAD \rightarrow FAD2H + NAD$.
- Hypoxanthine $\xrightarrow[\text{x. oxidase}]{\text{FAD}}$ xantinine + reduced FAD.2H.
- Oxidized FAD (yellowish), reduced form (colourless).

Structure: riboflavin (B2), 2 phosphoric acid, ribose and adenine.

FAD

CH_3 CH_3 — Ribitol-ph-ph-Ribose — adenine (NH_2)

- FMN: (Flavin mononucleotide):
  - Acts as hydrogen carrier from reduced NAD.
  - Structure: also as hydrogen carrier in resp. chain oxid. (Flavin-ribitol-phosphoric acid) = FMN.

4. Lipoic acid: (Is hydrogen carrier, vit. B complex member):

Act as hydrogen carrier in oxidative decarboxylation process.

$$\text{Pyruvate} \xrightarrow[\text{lipoic acid, CoASH}]{\text{TPP, NAD, FAD}} \text{active acetate.}$$

Oxidized lipoic acid is changes to reduced form

$$L\langle^S_S : \cdot CH_2 \cdot CH_2 \cdot CH \cdot (CH_2)_4 \cdot COOH \xrightarrow{2H} CH_2 \; CH_2 \cdot CH \cdot (CH_2)_4 \cdot COOH : L\langle^{SH}_{SH}$$

5. Co. enzyme Q:

Acts as hydrogen carrier in respiratory chain oxidation, and linke between FMN ano cytochrome b in respiratory chain.

$$FAD2H + \text{Coenzyme Q} \rightarrow \text{Co. } Q2H + FAD.$$

Structure: has relation to vit. K.

II. Co. enzymes act as acid group carrier = co. acetylase:

= CoA. SH:

Act as acetyl carrier (-COOH), which particepates in CHO, lipid, and protein metabolism.

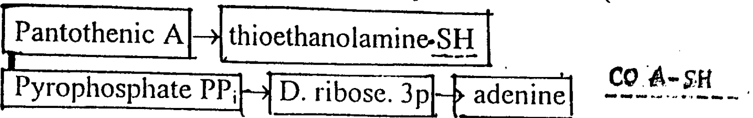
ACTIVE ACETATE

Acetic.A → $CH_3\ COOH + CoA\ SH \rightarrow CH_3.\ Co.\ SCoA + H_2O$

Acetic acid is the common pool of food staff metabolism

[$CH_3.CO.COOH$ (pyruvic acid) $\xrightarrow[\text{lipoic A, CoASH}]{\text{TPP, NAD, FAD}}$ active acetate ($CH_3.CoASH$).

Structure: β. Alanine+pantoic A $\xrightarrow{\text{ATP}}$ pantothenic acid (vit.B member).



III. Co. enzymes act as Co2. carrier, co. carboxylase = TPP

1. Thiamine pyrophosphate, vit. B_1):

- Acts as activator of decarboxylase enzyme, as in oxidative decarboxylation of pyruvate → acetate

  $CH3.\ CO.\ COOH \xrightarrow[\text{lipoic A, CoASH}]{\text{TPP, NAD, FAD}} CH3.\ CO.\ CoASH$ active acetate.

- So, if thiamin is deficient, pyruvate not converted to active acetate, leading to accumulation of pyruvate in blood, and impairing of complete CHO oxidation. And because nerve tissue depends upon CHO metabolism, it suffers and manifested as peripheral neuritis.

  Structure: (Vit. B_1+ PP_i) pyruvic attach here.

thiazolc.R

NH_2 — CH_3 — Thiamine — H

H_3C — $-CH_2-CH_2-O-$ — TPP

pyrimidine.R

- Insulin is needed for proper phosphorylation of vit. B1-→T.P.P.

2. **Biotin (vit. H):** acts as CO_2 carrier in carboxylation processes. Acts as coenzyme of carboxylase which fixes CO_2;

$$\underset{\text{PYRUVATE}}{CH_3.\ CO.\ COOH} \xrightarrow[\text{Biotin} + CO_2]{\text{Pyruvate carboxylase}} \underset{\text{OXALOACETATE}}{HOOC.\ CH_2.\ CO.\ COOH.}$$

Structure:

H₂C–S–CH ring fused with HC–CH and HN–C(=O)–NH; side chain: CH-CH2.CH2.CH2.CH2.COOH

BIOTIN

IV. Co. transaminase, amino group carriers and decarboxylation processes, PLP (pyrodoxal ph) vit. B6:

1. Acts in transferring of amino group ($CH\text{-}NH_2$) of certain aminoacids as alanine and aspartic acids to α. keto-acids as pyruvate, in presence OF certain enzymes as Got and Gpt.

- $CH_3\text{-}CH(NH_2)\text{-}COOH$ (Ala) + α CO·COOH, β CH₂, γ CH₂·COOH $\xrightarrow[\text{SGPT}]{\text{PLP}}$ PYRUVATE + GLU.A
- $H_2N\text{-}CH(COOH)\text{-}CH_2\text{-}COOH$ + α CO·COOH, CH₂, CH₂·COOH $\xrightarrow[\text{SGOT}]{\text{PLP}}$ oxaloacetate + GLU.A

aspartate α. keto glutarate

CHO, HO, CH3, N, $CH_2 - O - P(=O)(OH) - OH$

PLP

2. Takes part in decarboxylation processes of specific amino acids as tyrosine, histidine, and glutamate, changing that into the corresponding amines in presence of decarboxylase enzyme.

- HISTIDINE (imidazole ring $-CH_2 \cdot CH(NH_2)-COOH$) $\xrightarrow[\text{Hist. decarboxylase, } -CO_2]{PLP}$ HISTAMINE (imidazole ring $-CH_2 \cdot CH_2 \cdot NH_2$)

- GLU. Acid ($H_2N \cdot \gamma CH \cdot COOH$, βCH_2, $\alpha CH_2 \cdot COOH$) $\xrightarrow[-CO_2]{PLP}$ GABA ($H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$)

V. Coenzymes act as carrier of other groups:

1. Folic acid, carrier of one carbon atom group

$$\text{Nor-adrenaline} \xrightarrow[+CH_3]{FH4} \text{adrenaline.}$$

2. Cobalamine acts as methyl group carrier (vit. B12).

Takes part in methylation of homocystiene to methionine.

- HOMOCYSTIENE ($CH_2(SH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$) $\xrightarrow[\text{methionine synthase}]{\text{methyl } TH_4}$ METHIONINE ($CH_2(S \cdot CH_3) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$)

ENZYME SPECIFICITY

Each enzyme catalyzes one reaction only, or limited range of enzymatic reactions; so enzymes show a high degree of specificity for the substrate (as lipases act on lipids, but not upon proteins or carbohydrates).

The degree of specificity varies from one enzyme to another, it may be:

1. Absolute specificity:

- In which enzyme acts on one substrate only (one enzymatic reaction).
- Uricase acts only on uric acid → allantoin.
- Urease acts only on urea → ammonia.
- Pyruvate kinase acts only on ph. pyruvate $\xrightarrow[ADP]{-P}$ enol pyruvate+ATP.

2. Reaction specificity:

Enzymes of every six major classes, has specific reactions according to its, own class, away from other classes, (hydrolases act on all members of that class, but not on other classes as transferases).

3. Relative specificity:

Has lesser degree of specificity; (lipase, not act only on triglycerides, but also on phospholipid). Also, hexokinase acts upon ATP and glucose to provide glucose 6. p, this reaction can be repeated on fructose and mannose → fructose 6.p, but not on galactose (needs galactokinase only).

4. Group specificity:

The enzyme acts only on specific group of substrate, as carboxypeptidase; acts on carboxyl end of the polypeptide chain, aminopeptidase acts only on amine end of the chain.

aminopeptidase ↙↙ NH_3^+ ~~~~~~~ ↙↙ carboxypeptidase COO^-

5. Stereo specificity:

If there is more than one type or form of substrate (L and D. glucose).

- The enzyme acts only on one type of the isomers D or L form.
- Proteolytic enzymes in body (pepsin and trypsin), split peptides composed of L. amino acids, not D. form.
- Human α-amylase in body acts only on strach (α1-4 link-glucose) but not on cellulose (β1-4 link glucose).

6. Dual specificity:

Enzyme acts on two different substrates:

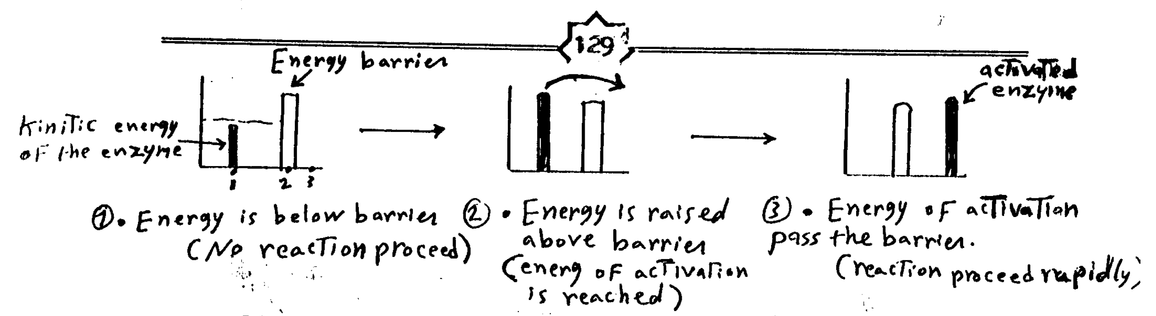
1- Xanthine oxidase oxidizes hypoxanthne $\xrightarrow{\text{X. oxidase}}$ xanthine.

2- Xanthine $\xrightarrow{\text{X. oxidase}}$ uric acid.

Initial energy of activation: (kinetic energy of the enzyme):

Molecules are in motion (some activity) at all temperature above the absolute zero (-273°C), but below that temperature, all molecular motion ceases.

In many reaction the molecules must posses kinetic energy ↗ enough to overcome the energy barriers of that reaction.



So, the kinitic energy (energy of activation) is the energy needed to overcome the energy barriers, and required in small amount only in the enzymatic reaction, or as boulder on hill, needs small energy to slipp down rapidly.

Mode of the enzyme action:

Enzyme can acts through the energy of activation, which activates the substrate without needing of high temperature or long time to perform it.

As: sucrose + sucrase enzyme → sucrose enzyme complex → enzyme + end product (glucose and fructose).

So, sucrose can be hydrolyzed with sucrase enzyme through the energy of activation of the enzyme which is needed only in small amount, without needing of high temperature or long time to perform it.

Classification of Enzymes

Enzymes are classified according to the type of reaction into six main groups in this order.

I. Oxidoreductases:

Which catalyse oxidoreduction processes.

i. Oxidation processes by:

a) Adding oxygen: Formaldhyde (H.CHO $\xrightarrow[HoH]{FH4}$ H. COOH formic acid.

LACTATE PYRUVATE

b) Removal hydrogen: CH_3. CHOH. COOH $\xrightarrow[LDH]{NAD}$ CH_3. Co. CooH + NAD_2H

c) Removal electron: reduced ferrous Fe^{++} $\xrightarrow[oxidation]{-e}$ Fe^{+++} oxid ferric.

ii. Reduction process:

As removal oxygen: $2H_2O_2$ (toxic) $\xrightarrow[-O_2]{Catalase}$ 2 H_2O + O_2 or adding hydrogen:

CH3. Co. COOH $\xrightarrow[LDH]{NAD2H}$ CH_3. CHoH COOH + NAD.

II. Transferases:

Which transfer a part or group from one compound to another.

Types:

1. Transphosphorylases: ATP+glucose $\xrightarrow{Hexokinase}$ glucose 6.p+ADP.
2. Transmethylases: which transfer methyl group.
3. Transaminases: alanine + α. ketoglutarate $\xrightarrow[SGPT]{PLP}$ pyruvate + glutamate.
   NH_2
4. Transmidinases: (HN=C): arginine + glycine → glycocyamine + ornithine.
5. Transamidases.
6. Transulferases: homocysteine $\xrightarrow{SH}$ Serine.
7. Transacylases: as choline acetylase

CH_3. CO. SCOA + CH_2. CH_2.N. (CH_3) $\xrightarrow[acetylase]{choline}$ acetylcholine + Co A. SH
OH

Acetyl CO A Choline

8. Transglycosylases: as glycogen synthetase and branching enzymes.
9. Transketolases: in pentosis metabolism.
10. Transaldolases: in pentosis metabolism.

III. Hydrolases:

Which cleavage (breakdown) the link of substrate by adding water.

Types:

1. Glycosidases: sucrose $\xrightarrow[H, OH]{Sucrase}$ glucose + fructose.
2. Carboxyesterases: act on neutral lipids, acetyl choline, cholesterol ester.

$R_1 \cdot COOH.R_2 \xrightarrow[H, OH]{Lipase} R_2OH$ (glycerol) + $R_1 \cdot COOH$ fatty acids.

Acetyl choline $\xrightarrow[esterase\ H, OH]{Choline}$ choline + acetic acid.

3. Phosphotases: as glucose 6. phosphatase in liver.

glucose 6. p $\xrightarrow[H, OH]{Phosphatase}$ glucose + phosphoric acid.

4. Peptidases: as pepsin, trypsin and peptidases, act on peptide link:

(Co.NH) $\xrightarrow[H, OH]{Peptidase}$ -CooH + NH_2

5. Amidases: as glutaminase and urease

glutamine $\xrightarrow[H, OH]{glutaminase}$ glutamate + NH_3

urea $\xrightarrow[H, OH]{Urease}$ $2NH_3 + CO_2$

6. Amidinases: as arginase enzyme.

Arginine $\xrightarrow{Arginase}$ ornithine + urea.

IV. Lyases:

Act by removal of a part or group from one substrate without adding water.

$$H_2CO_3 \xrightarrow[\text{anhydrase}]{\text{Carbonic}} CO_2 + H_2O.$$

V. Isomerases:

Act by interconversion of a chemical group from one place to other in the same molecule.

Types:

1. Mutases: glucose 6.p $\xrightarrow{\text{glucomutase}}$ glucose 1.p.
2. Epimerases: UDP.galactose $\xrightarrow[\text{epimerase}]{\text{UDP galactose 4}}$ UDP. glucose.
3. Aldoketoisomerases: glucose 6.p $\xrightarrow[\text{isomerase}]{\text{phosph. glucose}}$ fructose 6. ph.

VI. Ligases:

Which link together two compounds.

1. Pyruvic acid+CO_2 $\xrightarrow[\text{biotin, ATP}]{\text{Pyruvate carboxylase}}$ COOH. CH_2. CO. COOH (OXALOACETATE)
2. CH3. COOH+CoASH (ACETIC ACID) $\xrightarrow[\text{ATP}]{\text{F.acid thiokinase}}$ CH_3.CO. SCoA+H_2O (ACTIVE ACETATE)

Other classification:

- Each enzyme has a specific number denotes its class, reaction, and name (enzyme commission) = E.C.
- Every enzyme has specific four numbers or digits denotes its class, reaction and name, as: LDH = 1.1.1.27 (four numbers) = E.C.

CH_3. CHOH. COOH + NAD $\xrightarrow{\text{LDH}}$ CH_3.Co. COOH + NHDH + H

LACTATE substrate — acceptor co-enzyme — PYRUVATE

I. The first number (1) of the enzyme denotes its major class (oxidoreductases).

II. The second number (1) of the enzyme (LDH), denotes subclass (name of substrate - alcoholic group. CHOH).

III. The third number (1), is sub. subclass, which is the acceptor (NAD) that remove or carry the 2H from substrate.

IV. The fourth number (27), denotes the specific name of enzyme catalyzing that reaction = LDH = 27.

Other examples:

ATP + glucose $\xrightarrow{\text{Hexokinase}}$ glucose 6.p + ADP.

Hexokinase = E.C = (2.7.1.1).

2 = second transferase class.

7 = subclass or the substrate = ATP

1 = sub. subclass, or the acceptor which carry the (ph) glucose.

1 = the name of this enzyme which catalyzes that reaction (Hexokinase) which is the first type in the transferase class.

FACTORS AFFECTING THE RATE OF ENZYME REACTIONS

1. pH (hydrogen ion concentration):

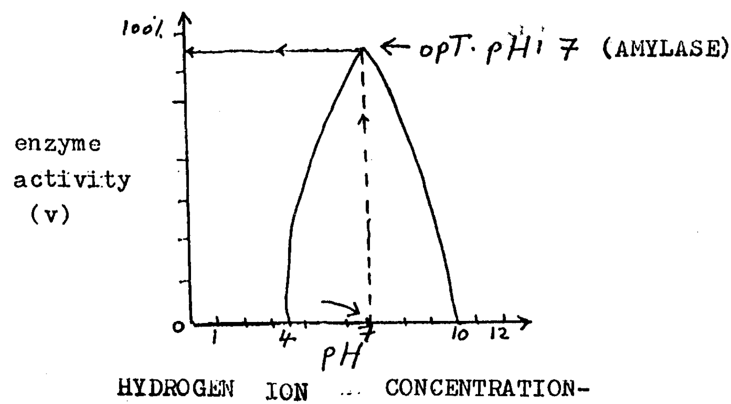
Definition:

Every enzyme has its optimum pH, at which its activity is at maximum.

When conc. of enzyme and substrate are kept constant, the reaction is augmented at certain pH value, but no reaction occurs at other pH values.

When enzyme activity is plotted against different pH values as in case of amylase enzyme opt. pH (7):

- In pH below 4 or above 9.5 no activity is observed, but maximum activity occurs at pH (7) which is called the optimum pH for enzyme activity.



Mechanism:

Each enzyme has its specific optimum pH, where the protein of the enzyme is in ionized state, and only one of the ionized protein is active.

So, any change from opt. pH by decreasing or increasing causes denaturation of its protein →inhibition of this ionized active form →decreasing enzyme activity.

2. Temperature:

Rate of any chemical reaction increases with elevation of temperature, every rise of 10°C will double the reaction rate.

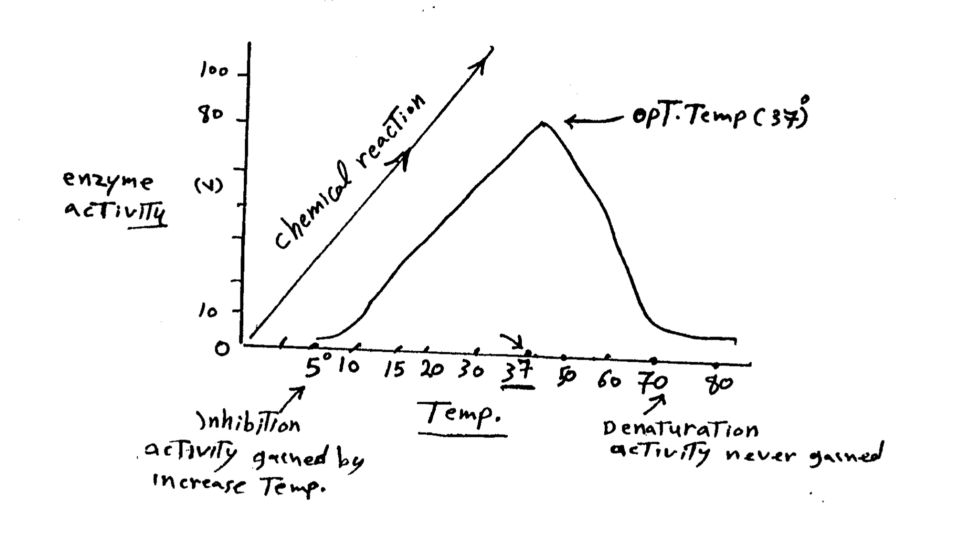
But in enzymes the initial reaction rate is also increased until certain temperature is reached (optimal temperature), in which any rise of temperature causes denaturation of enzyme proteins, which are completely inactivated above 70°C.

So, the optimum temp is the result of the balance between the rate of increase activity and the rate of enzyme destruction.

Also, the opt. temperature of any enzyme depends upon time or duration of exposure to temperature (the shorter the time of temp. exposure, is the higher in the opt. temperature).

- (Accumulation of end product → decreases enzyme activity).

$$\text{Temp. Coefficient } Q_{10} = \frac{\text{Volecity rate at } (T^\circ + 10^\circ)}{\text{Velocity at } T^\circ}$$



3. Concentration of the enzyme (substrate and other factors are fixed):

Definition:

Increase concentration of the enzyme leads to increase in the rate of enzymatic activity in a progressive pattern.

But deviation may occur from that activity due to accumulation of the end product, inhibitory factors, and substrate accumulation.

Explanation:

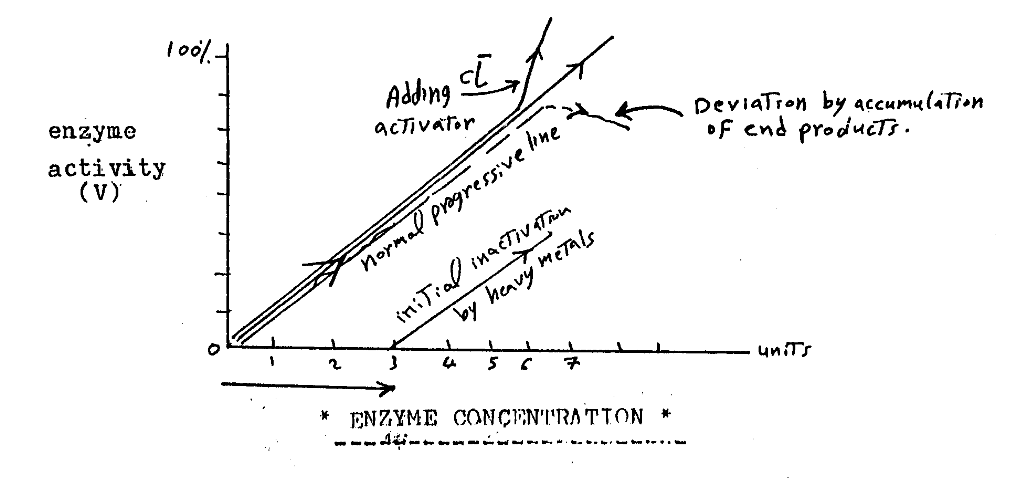
(E + S → ES complex → E + P (end product).

Activators:

If an activator is added (cl^-) as Nacl → more progressive reaction.

Inhibitors:

Initial velocity may be inhibited if heavy metals are added as lead, mercury, and arsenic (reaction begins late).



4. Substrate concentration:

Definition:

If the concentration of substrate is gradually increased in an enzyme reaction, and keeping all other factors constant (pH, temp., and ezyme concentration), the rate of enzyme velocity (v) will increase with addition of more substrate, until maximum velocity is reached (V_{max}).

Then any increase in substrate concentration cause no further increase in the reaction rate due to saturation of all active centers with substrates.

Explanation:

i. At low substrate concentration, the rate of reaction velocity (v) is increased in linear progressive pattern = (first order kinitics), due to presence of the active centres in enzymes.

ii. At moderate substrate concentration, the rate of reaction velocity (v) also increase, but in lower rate (not linear), so the curve begins to fall down until the maximum velocity rate (V_{max}) is reached.

iii. At high substrate concentration, any further addition cause no any increase in the reaction velocity (v) = (zero order kinitics).

This can be explained due to saturation or occupation of the enzyme active centres with the substrate, so no more (ES) complex will be formed, so no further reaction rate occurs.

So, the curve here is in form of hyperbola (the activity is limited, because the curve is not linear).

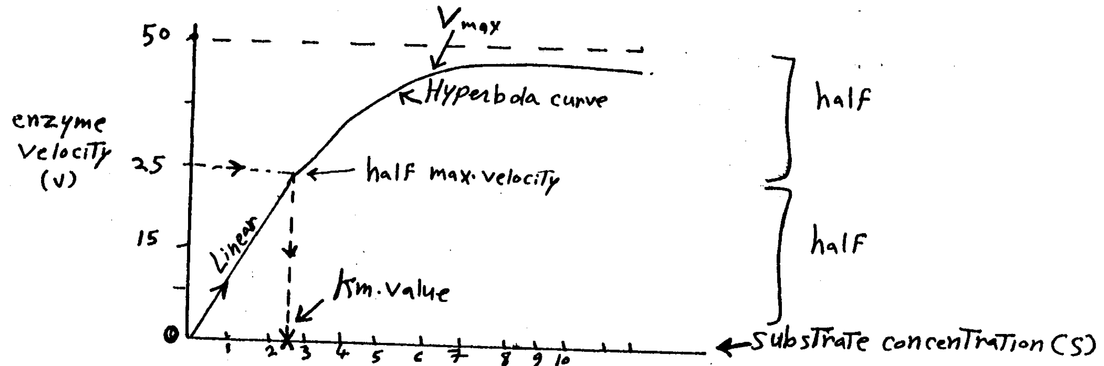
E + S → ES complex → E + P — All catalyzed

E + S → ES complex → E + P + S — not catalyzed

Result:

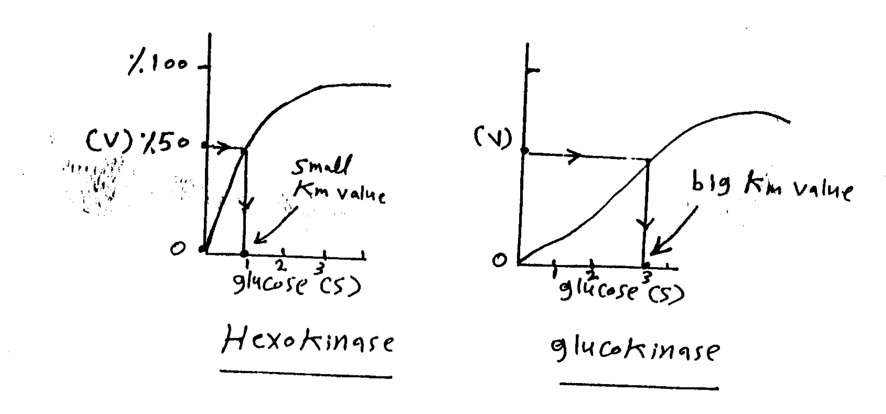
From the curve it is possible to deduce or conclude the concentration of substrate which is required to attain half maximum velocity, this value is called Michaelis constant or km. value.

$$\therefore K_m = \frac{[E_t - E_s] \times S}{E_S} \qquad \therefore E_t = \text{Total enzyme.}$$



The smaller the Km value, is the more active the eznyem; needs the smallest substrate concentration to attain 1/2 V max.

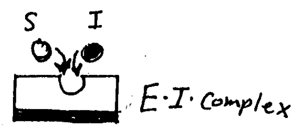
Hexokinase is more active than glucokinase, because it needs less substrate to reach 1/2 V max = small Km value.



5. Enzyme inhibitors:

Which cause depression in enzyme activity.

Types:



I. Competitive inhibition (reversible):

The inhibitor and substrate are similar in chemical structure, so they compete each other for occupying the active site of the enzyme.

The enzyme activity depends upon the relative concentration of inhibitor or the substrate.

The inhibition is reversible, if add excess substrate.

Example:

Sulpha and para-aminobenzoic acid (PABA); (has similar structure).

- Sulpha acts as inhibitor for the enzyme system essential for bacterial growth.
- PABA, acts as substrate of enzyme system of the bacteria.
- They have similar structure, so, they compete each other for the enzyme system of the bacteria.
- The activity of bacterial enzyme system depends upon relative concentration of sulpha or PABA.

II. Non-competitive inhibition (Irreversible):

In which there is perminant inhibition of the enzyme by denaturing it.

The inhibitor combines with the enzyme in site other than the active center, so enzyme can bind both inhibitor and substrate at the same

time, forming a firm enzyme substrate inhibitor complex which is unable to breakdown (irreversible).

S
I
E·S·I· complex

Examples:

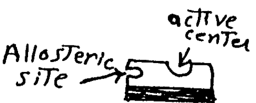
Heavy metals as silver, copper, cyanides, mercury, lead, heat and acids.

6. Enzyme activators:

Which activate the enzyme substrate complex as metal ions.

- Cl^- (Nacl): activate amylase.
- Ca^{++} : activate thrombokinase for clotting process.
- mg^{++} : activate alkaline phosphatase.

Allosteric regulation of enzyme activity:



Allosteric enzymes:

Some enzymes has other sites than the active centre to combine with other substances to activate the enzyme (effector) or to inhibit it (inhibitor), so react with additional substrates or substances.

Effect of substrate on allosteric enzymes:

- Most enzymes give a hyperbola curve, when velocity is plotted against substrate concentration as in Michaelis theory.
- But there is some enzyme not follow that theory (Allosteric enzymes), which give a sigmoid curve (rather than the hyperbola curve); S. shape.
- These allosteric enzymes are made up of subunits, and contain other active allosteric sites rather than the active centre of the enzyme.
- So, react with additional substrates.

Metabolic control:

Allosteric enzymes have specific sites for binding of activators or inhibitors, which cause a change in protein of the allosteric enzyme, and so affect the enzyme activity.

Allosteric inhibitors:

Citrate in kreb's CHO cycle is an allosteric inhibitor; when citrate production increases or accumulates due to increase rate of kreb's cycle oxidation, it inhibits the phosphofructokinase (key enzyme in control of glycolysis) to avoid the effect of increased kreb's cycle intermediates (prevent excess energy).

Allosteric activators:

ADP when increased acts as allosteric activators of phosphofructokinase →increase rate of glycolysis and CHO oxidation → increase energy production (ADP + Energy $\xrightarrow{+\sim P}$ ATP (energy donor).

Glucose
ph.fructokinase
pyruvate
acetate
Krebs
citrate
ADP.
Energy
(+)
(−)

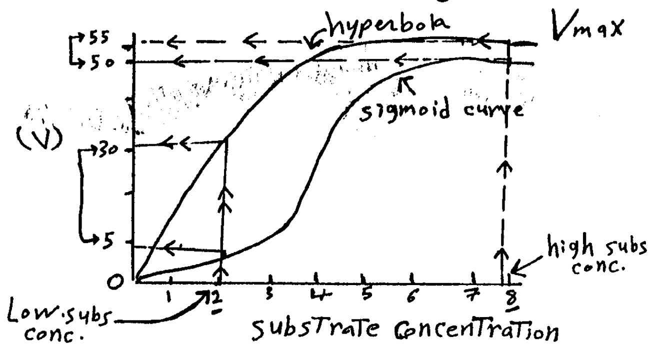
Importance of allosteric enzymes:

Relation between substrate concentration and allosteric enzymes:

i. At low concentration of substrate, the velocity rate (activity) is low relative to that in usual hyperbola curve (micheal's theory) due to partial inhibition of the allosteric enzymes (so, the difference in activity here is high).

ii. At higher concentration of substrate (increasing rate), the degree of inhibition of the allosteric enzyme is lessened due to stimulation of other allosteric sites. SO, at higher substrate concentration the difference in activity is low.

Therefore, the control of allosteric enzymes activity can be achieved by small changes in substrate concentration at this degree of high substrate concentration.



ISOZYMES

Isozymes are fractions or subtypes of one enzyme having the same catalytic activity, but differ in chemical and level of quaternary structure.

Example:

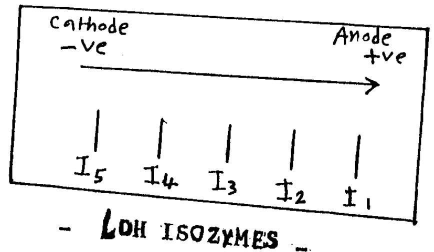
LDH (five isozymes), CPK (three isozymes), transaminases (2 isozymes; Gpt, Got) and phosphatases (2; Acid and Alk).

1. LDH:

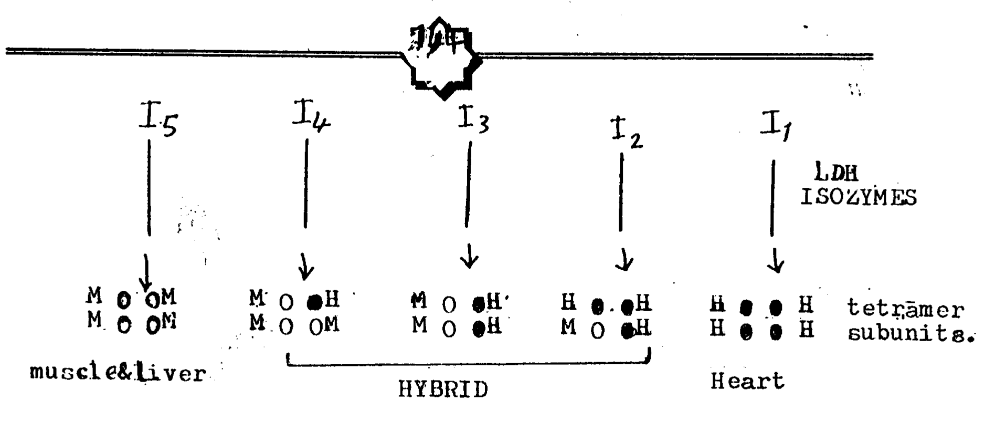
Location: Present in cytosole of tissue and cells of mammals, birds, insect and plants, also in plasma.

Structure:

- When LDH enzyme is subjected to electrophoresis, it separates into five units or fragments.
- They numbered according to the speed of migration to the anode (+ve) with electrophoresis. So that, LDH_1, is the fasted, and LDH_5 is the lowest in mobility.
- Each unit or fragment consists of four subunits (tetramer); four peptide chains.
- If they predominate in the heart, they named (HHHH) subunits as in LDH_1.
- If they predominate in the skeletal muscles and liver, they named (MMMM) subunits as in LDH_5.
- Isozymes 2,3, and 4 have varying proportions of both H and M subunits, and named hybrid subtypes of molecules, increase in myositis.



- LDH ISOZYMES -



- LDH SUBUNITS-

Importance: Detection of heart and liver diseases.

- Cardiac infarction: there is cell destruction and liberation of (LDH, mainly LDH_1 and LDH_2 used as diagnostic data in cardiac infarction, especially, in the first day.
- Liver diseases: LDH_5 is raised during clinical peak of acute hepatitis, (M) type.
- Also LDH, may generally raised in relapses of acute and chronic leukemia, generalized carcinomatosis, and pernicious anemia.

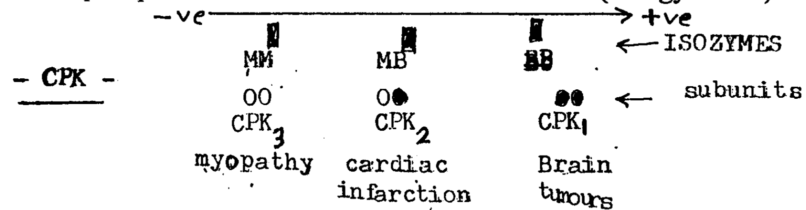
1	1	1	1	1	Normal
1	1	1	♦	♦	Cardiac infarction
♦	1	1	1	1	Acute hepatitis
I_5	I_4	I_3	I_2	I_1	

2. CPK: (creatine phosphokinase):

- CPK is composed of 3 units by electrophoresis.
- Each unit is subdivided into 2 subunits (M; muscle and B; brain).

- Creatine + ATP $\xrightarrow[\text{Transphosphorylase = cpk}]{\text{ATP creatine}}$ creatine phosphate + ADP.
- Creatine phosphate + ADP $\xrightarrow{\text{CPK}}$ creatine + ATP (energy donor).

- CPK -



3. Transaminases:

1. SGPT: increases in acute liver disease.

2. SGOT: increases in cardiac infarction.

4. Phosphatases:

i. Alkaline phsophatase: increases in rickets, obstructive; jaundice.

ii. Acid phosphatase: increases in cancer prostate.

Enzymes in clinical diagnosis:

I. Functional plasma enzymes:

- Enzymes that perform physiological role in the blood (has function).
- The substrate of enzyme is present in circulation; lipoprotein lipase acts upon chylomicrones (substrate). Its deficiency leads to primary hyperlipoprotienemia Type I(increase plasma triglycerides).

II. Non-functional plasma enzymes:

- Enzymes has no known physiological role in the blood.
- Their substrates are absent from circulation.
- The enzymes themselves are present in normal blood at very low level than in tissue cells.

- So, increasing the enzyme levels in the blood, suggest increase in tissues destruction (disease), so can used as diagnostic value in diseases.

Examples:

1. Pancreatic lipase and amylase: increase in acute pancreatitis.
2. Acid phsophatase: ↑ in cancer prostate.
3. $LDH_{1,2}$, CPK_2: ↑ in cardiac infarction.
4. Cholinesterase: ↑ in nephrotic syndrome.
5. Transaminases: ↑ in liver diseases.

Control of enzyme synthesis: (Enzyme induction and repression):

I. Enzyme induction:

Is increase in synthesis of an enzyme (its amount) in response to specific substance known as inducer.

Inducible enzymes; are that rapidly synthesized in increased amounts in response to the substrate (inducer) in the growth media.

Example:

- Escherichia coli (has many enzymes, but deficient in permease and β. galactosidase, whose transport the enzyme inducer lactose inside the E-coli, and aid synthesis of that enzymes (inducible enzymes) inside the (E-coli).

- So, when E-coli grow on media containing lactose from the start, it will induce the E-coli to produce the permease and a β-galactosidase by induction of its synthesis to ferment lactose.
- But, if lactose is not added to E-coli media → no lactose fermentation.
- Therefore, the lactose is called enzyme inducer, and produced enzymes are called (inducible enzymes).

II. Enzyme repressor:

Is decrease in synthesis of an enzyme in response to a small molecule known as co-repressor.

Mechanism:

The co-repressor is attached to specific protein in enzyme containing cell (E-coli) called Apo repressor, forming (co-repressor-Apo repressor complex), which cause repression in enzyme synthesis may through inhibition of mRNA.

When repressor is removed, this called derepression.

Production of enzyme synthesis (amounts) depends upon relative relation between induction and repression.

Vitamins

Key words

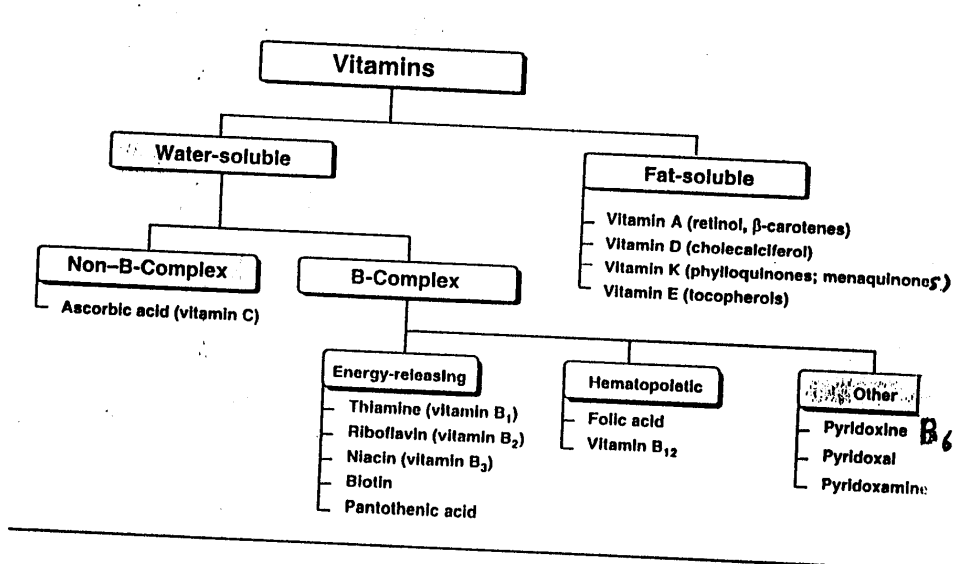
- Apoenzyme: A protein moiety of an enzyme that requires a coenzyme.
- Avitaminosis: A disease condition, described as a deficiency syndrome, resulting from lack of a vitamin.
- Coenzyme: An organic molecule, generally derived from a vitamin, that functions catalytically in an enzyme system.
- Cofactor: A natural reactant, usually either a metal ion or a coenzyme, required in an enzyme-catalyzed reaction.
- Holoenzyme: A catalytically active enzyme constituted by coenzyme bound to apoenzyme.
- Hypervitaminosis: An unhealthy condition resulting from excess of a vitamin.
- Vitamins: An essential organic micronutrient that must be supplied exogenously and in many cases is the precursor to a metabolically derived coenzyme.
- Vitamer: A term used to describe any of a number of compounds that possess a given vitamin activity.

Definition

- Vitamins are organic compounds required by the body in trace amounts to perform specific cellular functions.
- They can be classified according to their solubility and their functions in metabolism.
- Vitamins cannot be synthesized by humans, and therefore must be supplied by the diet.

Classification of vitamins:

- Nine vitamins (Thiamine; B_1, Riboflavin; B_2, Niacin, B_3; Biotin; H, Pantothenic acid; B_5; Folic acid, Cobalamine; B_{12}, Byridoxine; B_6 and Ascorbic acid, Vit. C) are classified as water soluble.
- Whereas four vitamins (vitamins A, D, E and K) are termed fat soluble, are more soluble in organic solvents, absorbed and transported with fat in diet.
- Water soluble vitamins function as coenzymes for numerous important enzymatic reactions, are not toxic due to their readily excretion in the urine, therefore they must be supplied continually in the diet.
- Whereas fat soluble vitamins are toxic if consumed in excess than recommended dietary intake, specially vit. A, D (its accumulating effect).



Classification of the vitamins.

I-WATER SOLUBLE VITAMINS

1) Thiamine (Vitamin B_1):

- *Chemistry*:
  - Thiamine consists of substituted pyrimidine ring attached to substituted thiazole ring through methylene bridge [CH_2], is sulphur containing vitamins.
  - Thiamine occurs in the cells as its active form TPP coenzyme in presence of ATP, and thiamine pyrophospho transferase.
  - Thisamine is a water soluble crystalline solid.
  - Is stable by acid media, but destroyed by alkali and U.V. (light), and thermostable.
  - Certain raw fishes contain thiaminase that destroy thiamine, so pathological deficiency symptoms can be developed in those conditions.

2-Methyl, 6-amino pyrimidine .

4-Methyl,5-hydroxy ethyl thiazole

Thiamine pyrophosphate (TPP) Co-Carboxylase

- *Sources*:
  - Present in high concentrations in yeast, liver, kidney, spleen, vegetables, and fruits.
  - Unrefined cereal grains, e.g., unpolished rice and bran.
  - Milk, egg, fish, and meat contain moderate amounts.

■ ***Biochemical role*:**

1. Thiamine pyrophosphate (TPP) is necessary for oxidative decarboxylation of (pyruvate $\xrightarrow{-CO_2}$ acetate), and α.ketoglutarate $\xrightarrow{-CO_2}$ succinyl CoA), which play a key role in energy metabolism of most cells, specially in nervous system, so in thiamine deficiency there is decreased production of ATP intracellular.
2. TPP is very important in transketolase reaction; transfer of ketol group
   CH_2OH
   |
   C = O
   as in hexose monophosphate shunt, so thiamine deficiency is diagnosed by an increase in erythrocyte transketolase activity observed upon addition of TPP.

- ***Deficiency of thiamine*:**

• Is due to poor food intake, or in areas where polished rice is the major component in the diet.

• Also in chronic alcoholism due to impaired absorption of thiamine, where the patients develop Wernicke-Korsakoff syndrome (apathy, memory loss, rhythical to-and-fro-motion of eyeballs)

• In thiamine deficiency in infants, they develop the Beri-Beri where suffer from tachycardia, vomiting, convulsions.

• In adults they suffer from dry skin, irritability, disordered thinking, and progressive paralysis.

- ***Methods of determinations*:**

1) By fluorometer or electrophoresis, and HPLC techniques in blood or urine.

2) Also through transketolase activity in RBCs (its normal is 0.75 → 1.30 U/gm Hb), or in whole blood (150-200 U/L)

- *Daily recommended dose*:

  1-2 mg/day

2) Riboflavin (B_2):

- ***Chemistry & structure***:
  - Is yellowish compound formed by Flavin (isoalloxazine ring) attached through its nitrogen (9) to C_1 of D. ribitol
  - Riboflavin is relatively heat stable, but sensitive to light, by irradiation with U.V. or visible light → irreversible decomposition.
  - Stable in acid media, but destroyed by alkali.

Riboflavin —(ATP → ADP)→ Flavin mononucleotide —(ATP → PPi)→ Flavin adenine dinucleotide

Structure and biosynthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

- ***Biochemial role***:
  - Riboflavin is changed into Flavin Mono Nucleotide (FMN) during its absorption with aid of ATP and Flavokinase enzyme.
  - In most tissues cytoplasm specially intestine, liver, heart and kidneys reacts with another ATP to form the coenzyme FAD, with release of PPi.
  - T_3 and T_4 stimulates FMN and FAD formation.
  - FAD and FMN act as hydrogen carriers in oxido-reduction reactions, and in energy production via the respiratory chain.

- *Sources*:
  - Liver, kidney, heart, meat, fish, and eggs white.
  - Leafy vegetables and fruits.

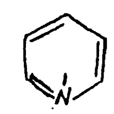
- *Deficiency*:
  - Sore throat, cheiolosis (dry fissured lip), angular stomatitis, glossitis (magenta tongue)
  - Seborrhoeic dermatitis of face.
  - Normochromic normocytic anaemia.

- *Reference intervals*:
  - 4 – 24 μg/dl in plasma or serum

- *Requirements*:
  - 1.6 – 2 mg/ day

3) Niacin (Nicotinic acid) – B_3

- *Chemistry*:
  - Niacin is a pyridine derivative that is a nontoxic component of the toxic alkaloid nicotine of tobacco.
  - Nicotinic acid and its amide; nicotinamide [contains an amide instead of carboxyl group] are the active forms of vitamin.
  - Niacin is heat stable, not sensitive to light, air, or alkali



Pyridine

COOH

Nicotinic acid

$CONH_2$

nicotinamide

- ***Sources***:
  - Nicotinic acid can be found in the body from the amino acid tryptophan (60 mg tryptophan gives rise to 1 mg nicotinic acid)
  - Tryptophan is present in meat, milk, eggs, and corn, but maize feeding (lack tryptophan) can lead to pellagra.
  - About 2/3 of niacin required by adults can be derived from tryptophan.

- ***Biochemical role***:
  - In cellular cytoplasm, niacin is phosphorylated by PP-ribose.p [1.pyrophosphate-ribose-5phosphate] to form NMN [nicotinate mononucleotide]
  - Then, $\text{NMN} + \text{ATP} \xrightarrow[+\text{ glutamine}]{-\text{Ppi}} \text{NADP}$ [nicotinamide adenine dinucleotide]

NAD

NADP

- NAD may be phosphorylated to NADP
- NAD and NADP act as hydrogen and electron carriers coenzymes in oxido-reduction reactions for energy releasing.
- The pyridine ring (of NAD or NADP) is the active center which accepts one hydrogen atom at C_4 to form the reduced form which is affected by acid media, while the oxidized form is affected by alkali.

H
$CONH_2$
Adenine
Ribose-Ⓟ-Ⓟ-Ribose
NAD^+
$:H^-$ Hydride ion
H H
$CONH_2$
Adenine
Ribose-Ⓟ-Ⓟ-Ribose
NADH

Reduction of NAD^+ to NADH.

- Niacin at doses 1.5 gm/day strongly inhibits lipolysis in adipose tissues → decrease free fatty acids which is used by the liver for triacylglycerol synthesis → decrease VLDL and in turn decrease LDL
- So, niacin is particularly useful in treatment of hyperlipoproteinemia type II b.

■ ***Deficiency***:

- Deficiency of niacin causes pellagra which shows dermatitis, diarrhea, dementia, and if untreated, death.
- Glossitis, and stomatitis.
- In dogs nicotinic acid deficiency may cause black tongue disease with viscous saliva.

■ ***Excretion***:

- Nicotinic acid is excreted in urine as methyl-nicotinamide (2.4-6.4mg/day)

■ ***Requirements***:

- 15 – 20 mg/day

4) Pyridoxine, B_6

- ***Chemistry***:
  - Pyridoxine is derivative of pyridine ring.
  - Pyridoxine, pyridoxal, and pyridoxamine are three natural forms of Vit.B_6, all are equally active.
  - Pyridoxine is absorbed from intestine, and in cellular cytoplastm it is converted to the active form pyridoxal phosphate [PLP] in presence of ATP and pyridoxal kinase enzyme.

Pyridoxamine   Pyridoxine

Pyridoxal   Pyridoxal phosphate

CONHNH2

N

Isoniazid

- ***Sources***:
  - Meat, fish, yeast, grains, seeds, and poultry.

- ***Biochemical importance***:
  - In body pyridoxal phosphate functions as coenzyme for large number of enzymes, specially related to amino acids

  1) Transamination:

  Oxaloacetate + glutamate $\xrightarrow[GOT]{PLP}$ aspartate +α. Ketoglutarate

2) deamination:

Serine $\xrightarrow[-NH_2]{PLP}$ pyruvate + NH_3

3) decarboxylation:

Histidine $\xrightarrow[-CO_2]{PLP}$ Histamine + CO_2

Glutamate $\xrightarrow[-CO_2]{PLP}$ γ. Aminobutyric acid [GABA]

4) condensation:

glycine + succinyl CoA $\xrightarrow{PLP}$ δ-aminolevulinic acid.

- ***Deficiency***:
  - In infants convulsions may occur due to deficiency of GABA which is C.N.S. inhibitor.
  - In adults sideroblastic anaemia (microcytic RBCs, with high serum iron) may result from decrease in Hb synthesis.
  - In some patients suffering from T.B., and receiving isoniazid, vit. B_6 is decreased, so pyridoxine should be taken daily (5-50 mg/day) with the T.B. drug.
  - Pyridoxine deficiency can lead to niacin deficiency, because PLP is needed for niacin synthesis from tryptophan.
  - In renal failure, pyridoxal kinase is inhibited → ↓ PLP in spite of adequate intake of pyridoxine.
  - Pyridoxine is required for conversion of homocystiene → cystiene, so its deficiency leads to hyperhomocystiemia which is a risk factor in cardiovascular diseases.
  - Pyridoxine is required for neurotransmitter synthesis:

Tryptophan $\xrightarrow[PLP]{-CO_2}$ serotonin

Tyrosine $\xrightarrow[PLP]{-CO_2}$ Norepinephrine

- So, its deficiency explains irritability, nervousness, and depression in mild pyridoxine deficiency.
- In severe cases peripheral neuritis and convulsions are developed.

■ ***Requirements***:

- 1.5 – 2 mg/day

5) Biotin (Vit. H)

■ ***Chemistry***:

- Biotin is sulfur containing vitamin.
- It is formed by union of imidazole and thiophene rings, which is attached to valeric acid.
- Biotin in liver and muscles is converted to its active coenzyme form biocytin.
- Biocytin acts as coenzyme for carboxylation (fixation of CO_2) and decarboxylation [removal of CO_2] reactions.

Thiophene
Imadizole
$-(CH_2)_4-COOH$
valeric acid

Biotin

COOH
α CH-NH$_2$
β CH$_2$
γ CH$_2$
δ CH$_2$
ε CH$_2$
NH
CH-$(CH_2)_4$CO

Biocytine(coenzym R)

- ***Sources***:
  - Liver, kidney, yeast and milk.

- ***Biomchemical role***:
  - Carboxylation reactions:

1) Acetyl CoA → Malonyl CoA

$$CH_3.\overset{\overset{O}{||}}{C}\sim SCoA \xrightarrow[\text{Carboxylase, Biotin, mg}^{++}]{CO_2,\ ATP \rightarrow ADP + Pi;\ \text{Acetyl CoA}} HOOC.CH_2.\overset{\overset{O}{||}}{C}\sim ScoA$$

Acetyl CoA   Malonyl CoA

Which is first step in fatty acid synthesis.

2) Pyruvic acid → Oxaloacetate

$$CH_3\text{-}CO\text{-}COOH \xrightarrow[\text{Carboxylase } {}^{+}\text{Biotin, mg}^{++}]{CO_2,\ ATP \rightarrow ADP + Pi;\ \text{Pyruvate}} \begin{matrix} COOH \\ | \\ CH_2.CO.COOH \end{matrix}$$

Pyruvic acid   Oxaloacetate

Which is important step for glucose oxidation in citric acid cycle.

3) Ammonia → Carbamoyl.ph

$$NH_3 \xrightarrow[\text{Synthetase, mg}^{++} + \text{Biotin}]{CO_2 + ATP \rightarrow ADP + Pi;\ \text{Carbamoly.ph.}} H_2N\text{-}COO\sim P$$

Ammonia   Carbamoyl.ph

Which is important step in urea Kreb's cycle formation.

- ***Deficiency***:
  - May be seen after ingestion of large amounts of raw egg white [containing avidin) which prevent absorption of biotin.

- Symptoms include: anorexia, nausea, vomiting, glossitis, pallor, depression and dry scaly dermatitis.

- ***Requirements***:
  - Biotin is synthesized by intestinal bacteria, so its deficiency is rarely seen.

6) Pantothenic Acid (Vit. B_5)

```
                                       β       ∝
H2 CH3OH O                    H       H2      H2
|γ  | β |∝ ||                 |       |       |
C — C — C — C  ----  N  —  C   -  C   - COOH
|   |   |
OH CH3  H
     ↑                                 ↑
Pantoic acid     +    B' alanine
--------------------------------
        Pantothenic acid
```

- ***Chemistry***:
  - It is formed from:
    i) β-alanine
    ii) Pantoic acid (α, γ dihydroxy, β dimethyl butyric acid)
  - It is viscous yellow oil, heat labile
  - It is readily absorbed and phosphorylated by ATP → 4.phospho-pantothenic acid.
  - Then cystiene is added → 4.phosphopantetheine
  - Phosphopantetheine is adenylylated by ATP → dephospho CoA
  - Dephospho CoA reacts with another ATP → CoA which is the active form of pantothenic acid.
  - CoA participates in CHO, lipid, and protein metabolism and acts as carrier and activation of acyl groups to extracts energy.

$$CH_3.COOH \xrightarrow{CoASH} CH_3.CO\sim scoA + H_2O$$

Acetate — Active acetate

- ***Sources***:
  - Liver, kidney, heart, meat, fish, and yeast

- ***Deficiency***:
  - Rare in human, but may irritability, hypotension, numbness, epigastric distress and constipation.

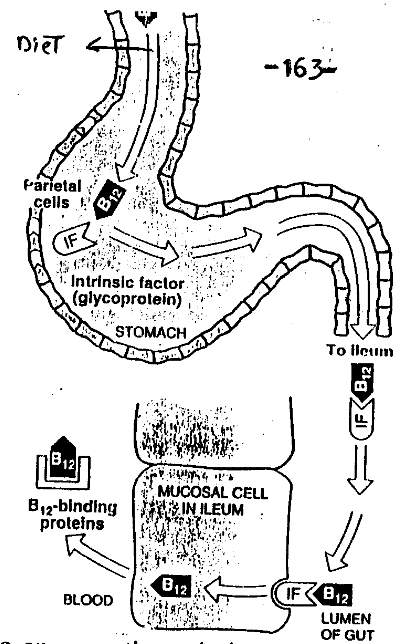
- ***Requirements***:
  - 10 – 15 mg/day

7) Cobalamin (Vit. B_{12})

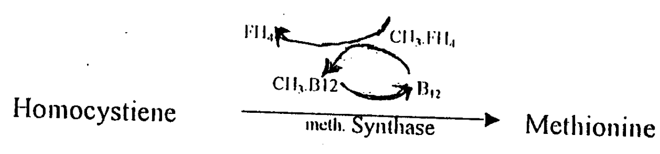
- ***Chemistry***:
  - Cobalamin consists of a porphyrin like ring, containing 4 pyrrol rings known as corrin ring.
  - Cobalt is attached to the four pyrrols via its nitrogen atoms.
  - Vit. B_{12} is attached to α nucleotide side chain.
  - Vit. B_{12} is heat stable, but destroyed by alkali, it forms dark red, needle like crystals.
  - Cobalt of Vit. B_{12} may attached to:
    1) Adenosyl group → Adenosyl cobalamin (in cytosols of liver, RBCs, and kidney), or
    2) Methyl group → methyl cobalamin (serum) or
    3) Cyanide group → Cyanocobalamin, [commercial form], and lastly
    4) Hydroxyl group→ hydroxy cobalamin, in body fluids and tissues)

- **_Sources_**:
  - Only animal sources; liver, kidney, meats, milk, and eggs.
  - It is not found in vegetables.
  - Bacterial synthesis in human colon, but it is not absorbed.

- **_Biochemical role_**:
  - Vit. B_{12} binds to intrinsic factor (IF) which is a glycoprotein secreted by the parietal cells of the stomach → Vit. B_{12} – IF complex.
  - In distal ileum, it bounds by receptors on mucosal cells to enter inside, where it is dissociated and passes into portal vein to liver cells
  - It is stored in the liver (about 1 mg), which cover the daily requirements for 2000 days, so Vit. B_{12} deficiency does not become evident for 5 years or more.



- Vit. B_{12} acts as co-enzyme through its four forms; methyl, hydroxy, cyano, and adenosyl cobalamins.
- The active form of Vit. B_{12}; (methylcobalamin) acts as coenzyme which participates in formation of methionine from homocystiene, in presence of methionine synthase, and methyl tetrahydrofolate ($CH_3.FH_4$). IN Vit B_{12} deficiency $CH_3.FH_4$ is **trapped** (not changed to folate) and called folate trap.



- Cobalamin is required for synthesis of the tetrahydrofolate active forms needed in purine and thymine synthesis, so its deficiency → megablastic anemia.
- Homocystiene is increased in Vit. B_{12} deficiency, and this in turn may participate in coronary heart diseases (homocystienemia may cause degradation of basement membrane of arterial wall → atheroma
- Deoxy adenosyl cobalamin acts as coenzyme which catalizes formation of succinyl CoA from methyl malonyl CoA in presence of adenosyl cobalamin CoA mutase.

Methylmalonyl CoA $\xrightarrow[\text{Adenosyl cobalamin mutase}]{\text{Adenosyl } (B_{12})}$ Succinyl CoA

- This reaction is the key step for catabolism of some branched amino acids (valine & isolucine) and odd number fatty acids.
- In deficiency of Vit. B_{12}, methyl malonyl CoA is increased, which interfer with myelination of nervous tissues → neurological disorders.
- Osteoblast activity is affected by B_{12} deficiency, and because osteocalcin (bone specific protein) is synthesized by osteoblast, so B_{12} deficiency may lead to decrease production of osteocalcin, which may participate in osteoporosis, specially in older people (above 60 years) where intrinsic factor and HCl production are decreased in stomach, due to atrophy of mucosa, so treatment in this condition is via B_{12} injection.

- ***Deficiency*:**
  - Is rare, but in absence of intrinsic factor pernicious anemia is developed.
  - Pernicious anemia is most common in young black females, or in middle-aged to elderly whites.
  - Vit. B_{12} deficiency may occur many years after partial gastrectomy, or ileal resection.
  - In older people above 60 years due to atrophy of gastric mucosa.
  - Also, Vit. B_{12} deficiency may be seen in vegetarians.
  - Pernicious anemia is associated with megalolastic anemia, leucopenia, and thrombocytopenia.
  - C.N.S manifestations as neuropathy, loss sensation in extremities, weakness, confusion, disoreintation, and dementia.
  - Folic acid should not be used in treatment of Vit. B_{12} deficiency, because temporary improvement is seen, followed by relapse and aggravation of C.N.S lesions.
  - Daily loss: 3- 4 μg/day, mainly through bile.

- ***Requirements***:
  - 5 – 6 μg/day
- ***Normal serum range***:

  (200 – 900pg/ml) mainly to serum protein

8) Folic Acid (Vit. M)

- Folate is found firstly in spinach leaves, and fresh green leafy vegetables (Folium = leaf), also found in potatoes, yeast, and meat organs.

- ***Chemistry***:
  - Folic acid contains in its structure:
  - i) Pteridine ring
  - ii) Para amino benzoic acid (PABA)
  - iii) One or more of glutamic acid; (one, three or seven moles of glutamate such as Pteroyl hepta glutamate)

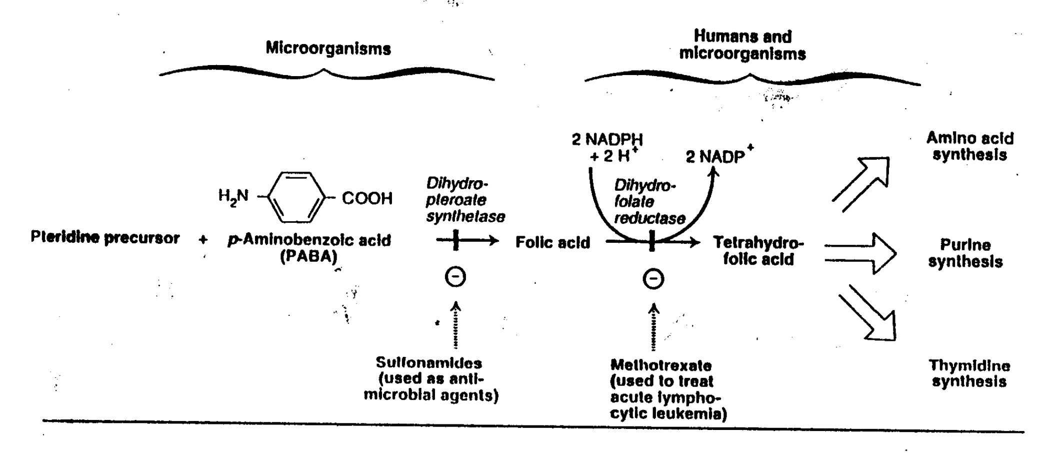
H_2N — Pteridine (OH) — CH_2 — HN — PABA — CO — [HN-CH(COOH)-CH_2-CH_2-COOH]$_n$

Pteridine PABA glutamic acid

Pteroic acid

- Folic acid in plants is present in the form of pteroyl heptaglutamate which is rapidly hydrolysed by intestinal folyl polyglutamate hydrolases into pteroyl monoglutamate which is readily absorbed.

- In body, folic acid is reduced by dihydrofolate reductase, and reduced NADPH + H^+ to dihydrofolic acid (this step is inhibited by sulfonamide drug). Then dihydrofolic acid is converted into tetrahydrofolic acid (H_4F) by another dihydrofolate reductase and 2 NADPH + H^+ (this step is inhibited by methotrexate which is used to treat acute lymphocytic leukemia).



Inhibition of tetrahydrofolate synthesis by sulfonamides and methotrexate.

- Most of pteroyl monoglutamate is reduced to tetrahydrofolate (H_4F) and methylated to N^5-methyl-H_4F within the intestinal cells.
- In blood stream, 2/3 of folate is bound to a protein, which may reduce folate loss from renal filtration.
- Folic acid play a key role in one-carbon metablism.
- Tetrahydrofolic acid (H_4F) functions as a coenzyme in transfer and utilization of one carbon fragments; the one carbon moiety may be in the form of:

  i) Methyl group ($-CH_3$) for active methionine, ii) methylene ($-CH_2-$) for thymidine, iii) methenyl ($-CH=$) for C8 purine, iv) formyl ($-CHO$) for C_2 purine, and v) formate (H.COOH) group.
- These one carbon fragments become attached to the nitrogen atoms number 5, 10 in (H_4F) mole

- Cooking destroys 50-90% of the folates present in the food.

- ***Deficiency***:
  - May result from:
  a) Insufficient dietary intake.
  b) Poor intestinal absorption (sprue)
  c) Excessive demands (as in pregnancy, liver diseases)
  d) Administration of antifolate drugs (as methotrexate), or anticonvulsant drugs.
  - Effects of folate deficiency results in:
  1) Inhibition of DNA synthesis due to decrease of purine, and d TMP synthesis → inhibition of RBCs maturation → abnormal large macrocytic RBCs with fragile membrane → macrocytic anemia.
     - Also there is megaloblastic changes in bone marrow, leucopenia, thrombocytopenia.
  2) Hyperhomocystienemia specially in eldery people may result in induction of atherosclerosis.
  3) Inadequate folate levels during first trimester, may increase the risk of neural tube defects → spina bivida in neonate.
  4) In folate deficiency, the feature is similar to that in B_{12} deficiency (macrocytic anemia) in which serum folate is normal in B_{12} deficiency.
  5) Diarrhea, and glossitis due to impairment of multiplication of alimentary tract epithelium.

- ***Folic acid antagonists***:
  - These are drugs used in treatment by inhibition of nucleic acid synthesis and cell division.
  - They have structure very similar to that of folic acid.

- In tissues they compete with folic acid, and thus interfer with nucleoprotein synthesis and cell division.

Examples:

1) Aminopterin (4.amino Folic Acid)
2) Amethopterin (4.amino-10-methyl Folic Acid) which inhibits folate reductase in mammalian cells and bacteria.
3) Dimethoprim; inhibits only folate reductase enzyme in bacteria, thus it is used as antibiotic.

- ***Normal value***:
  - 3 20 ng/ml in serum

- ***Requirements***:
  - 0.4 – 0.8 mg/day

9) L. ascorbic acid (Vit. C)

L-gulonic acid | L-Ascorbic acid ⇌ (−2H / +2H) Dehydro-L-Ascorbic acid

- *Chemistry*:
  - Vitamin C is derivative of sugar acid monosaccharide known as L.gulonic, which is derived from glucose during uronic acid pathway.

- L. ascorbic acid is easily oxidized to its another active form dehydro L-ascorbic acid.
- Vitamin C is water soluble; destroyed by heat.
- Ascorbic acid is reducing agent, acts in important hydroxylation reactions in the body; hydroxylation of lysine and prolane in procollagen → normal collagen fibrils.
- The acidity of L-ascorbate is due to presence of a dienol group in its structure - C = C- , in which its hydrogen dissociate to give hydrogen

  | |

  OH OH

  ion which cause its acidity.
- Vitamin C is present in active L.form, but D-form has no biological activity.

■ ***Sources***:

- Citrus fruits, melons, Guava, fresh green vegetables as lettuce, green pepper, and tomatoes.
- Vitamin C is synthetized by many plants and animals except humans and guinea pigs [lack of gulonolactone oxidase which form L.ascorbic acid], so Vit. C should be supplied in diet.
- Milk is poor in Vit. C, so infant should be given fruit juices by age of 3-6 months.

■ ***Biochemical function***:

- Vitamin C is important for maintenance of normal connective tissues, and wound healing due to formation of normal collagen.
- Formation of intercellular matrix of bone, cartilage, teeth, and capillary wall.
- Enhances absorption of iron in ferrous state, so aids in formation of blood hemoglobin.

- Vitamin C is concentrated in glandular tissues, such as adrenal cortex, pituitary, corpus luteum, thymus, and retina which has 20-30 times than plasma concentration.
- Vitamin C acts as Co-factor in hydroxylation reactions in corticosteroid synthesis during stress.
- Vitamin C is involved in synthesis of epinephrine and anti inflammatory steroids by the adrenals through tyrosine metabolism.
- Vit. C is needed for hydroxylation of the betaine of butyric acid to form carnitine which promotes oxidation of long chain fatty acids.
- Vit. C is involved in reduction of folic acid to active tetrahydrofolate (H_4F)
- Vit. C is one of antioxidants which inactive the toxic oxygen free radicle which may cause damage to cell membrane, and cellular DNA, free radicles may cause cancer, heart and lung diseases and even aging.

■ ***Deficiency***:

- Prolonged deficiency of Vit. C leads to scurvy which characterized by:
  - Weakness of capillary wall → bleeding under skin (petichae) specially in lower thighs and buttocks.
  - Swollen tender gum, and teeth become loose.
  - Delay in healing of wounds, may bone fractures due to deficiency in collagen formation.
  - Ocular hemorrhages, drying of salivary and lacremal glands.
  - Anemia may develop due to decrease utilization, and absorption of iron.

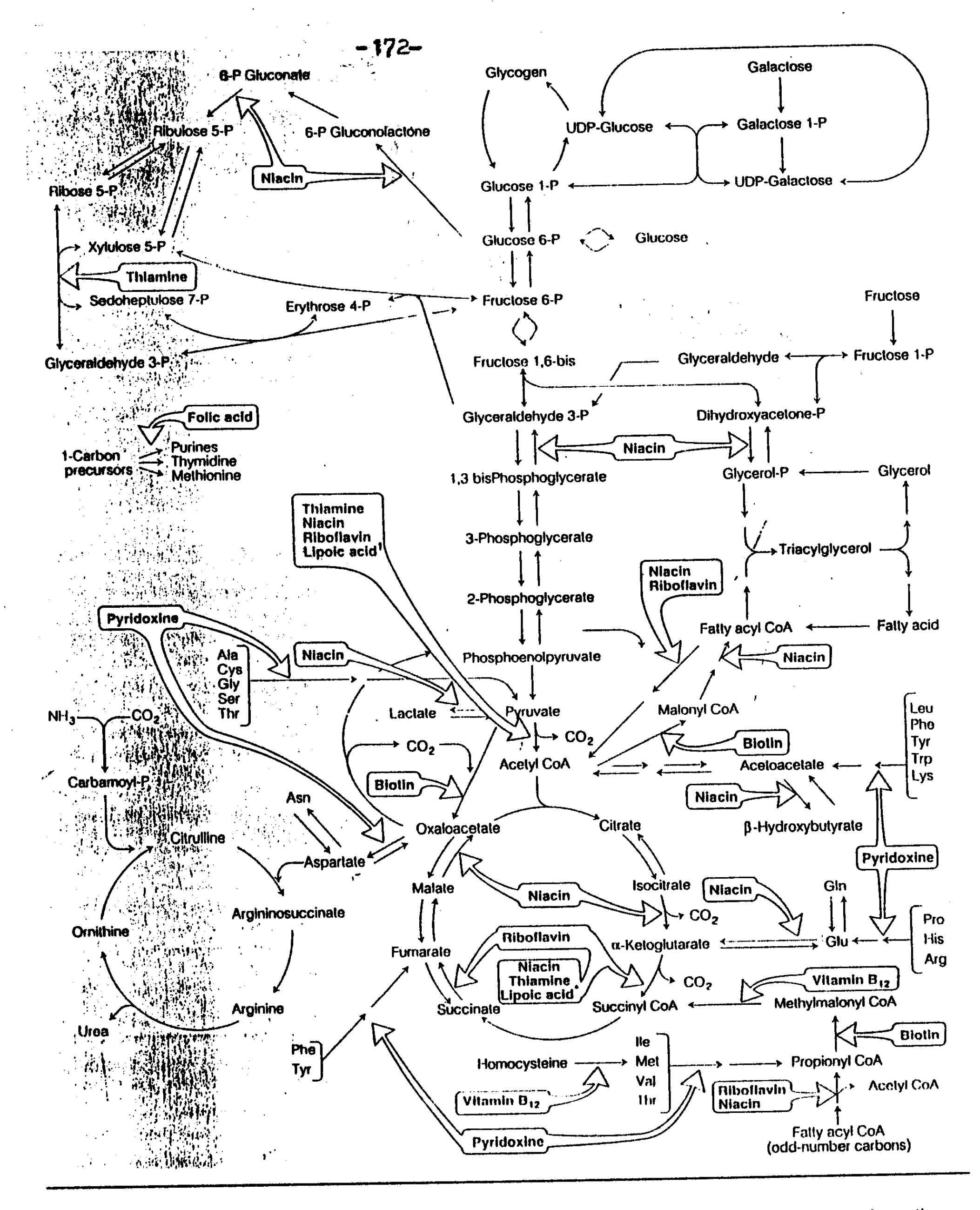
■ ***Toxicity of ascorbic acid***:

- Mega doses of vitamin C is generally non toxic, however, may production of oxalates which may deposit in kidney or bladder.

- The oxidized form of ascorbic acid (dehydroascorbic acid) is toxic, so high doses of Vit. C could favor the accumulation of dehydroascorbate specially if there is defect in enzyme system which reoxidizes dehydroascorbate to ascorbate.

- ***Normal serum value***:
  - 0.3 – 2 ng/dl in serum, below 0.2 ng/dl → scurvy.

- ***Requirements***:
  - 50 – 100 mg/day.



Summary of some reactions involving coenzymes derived from some water-soluble vitamins. Curved reaction arrows (⟲) indicate that the forward and reverse reactions are catalyzed by different enzymes. (*Lipoic acid is not classified as a vitamin, but it is a coenzyme involved in energy metabolism.)

Vitamins Required by Humans*

Common Name	Trivial Chemical Name	General Roles	Symptoms of Deficiency or Disease	Direct and Indirect Assays
Fat Soluble				
Vitamin A_1	Retinol	Vision, growth, reproduction	Nyctalopia, xerophthalmia, keratomalacia	Photometric, fluorometric, dark adaptation, RIA, HPLC
A_2	3-Dehydroretinol			
Vitamin D_2	Ergocalciferol	Modulation of Ca^{+2} metabolism, calcification of bone and teeth	Rickets (older infants and children), osteomalacia (adult)	CPB, HPLC, RIA
D_3	Cholecalciferol			
Vitamin E	Tocopherols, α, β, γ, δ Tocotrienols	Antioxidant for unsaturated lipids	Lipid peroxidation, including red-blood cell fragility, hemolytic anemia (premature, newborn)	Photometric, HPLC, erythrocyte hemolysis
Vitamin K_1	Phylloquinones	Blood clotting, osteocalcins	Increased clotting time, hemorrhagic disease (infant)	Photometric, HPLC, prothrombin time, RIA (abnormal prothrombin)
K_2	Menaquinones			
Water Soluble				
Vitamin B_1	Thiamine	Carbohydrate metabolism, nervous function	Beriberi, Wernicke-Korsakoff syndrome	Fluorometric, microbiological, transketolase, HPLC
Vitamin B_2	Riboflavin	Oxidation-reduced reactions	Angular stomatitis, dermatitis, photophobia	Fluorometric, HPLC, microbiological, glutathione reductase
Vitamin B_6	Pyridoxine, pyridoxal, pyridoxamine	Amino acid, phospholipid, and glycogen metabolism	Epileptiform convulsions, dermatitis, hypochromic anemia	Microbiological, HPLC, tyrosine decarboxylase
Niacin	Nicotinic acid	Oxidation-reduction reactions	Pellagra	Microbiological, fluorometric
Niacinamide	Nicotinamide			
Folic acid	Pteroylglutamic acid	Nucleic acid and amino acid biosynthesis	Megaloblastic anemia	CPB, microbiological, immunoassay
Vitamin B_{12}	Cyanocobalamin	Amino acid and branched-chain keto acid metabolism	Pernicious and megaloblastic anemia, neuropathy	CPB, microbiological, immunoassay
Biotin	—	Carboxylation reactions	Dermatitis	Microbiological, photometric, carboxylases, avidin binding
Pantothenic acid	—	General metabolism	Burning feet syndrome	Microbiological, photometric, enzymatic, HPLC-avidin binding, CPB/HPLC
Vitamin C	Ascorbic acid	Connective tissue formation	Scurvy	Photometric, HPLC

RIA, Radioimmunoassay; *HPLC*, high-performance liquid chromatography; *CPB*, competitive protein binding.
*Only one vitamer from a vitamin group (for example, A_1 for vitamin A or pyridoxine for vitamin B_6) is sufficient for that vitamin.

II-FAT SOLUBLE VITAMINS

- Vitamins; A, D, E, and K are termed as fat soluble vitamins.
- They need bile salts for absorption, and transported with fat of the diet in the chylomicrons lipoprotein to the liver.
- Are derived from the hydrophobic unsaturated hydrocarbons known as isoprene.
- They are not excreted in urine, but in bile, and stool, so excess of Vit. A and D in diet lead to toxic effect due to accumulation in liver and adipose tissue.

Vitamin A (Retinol)

B Carotene

O_2 Carotenase (dioxygnase)

$(CH = CH - C(CH_3) = CH)_2-CHO$

Retinal (Vit A aldehyde)

$NADH + H^+$ → NAD^+ alcohol dehydrognase

$(CH=CH - C(CH_3) = CH)_2-CH_2OH$

retinol (Vitamin A_1)

$(CH = CH - C(CH_3)=CH)_2-CH_2OH$

retinol$_2$ (Vit A_2)

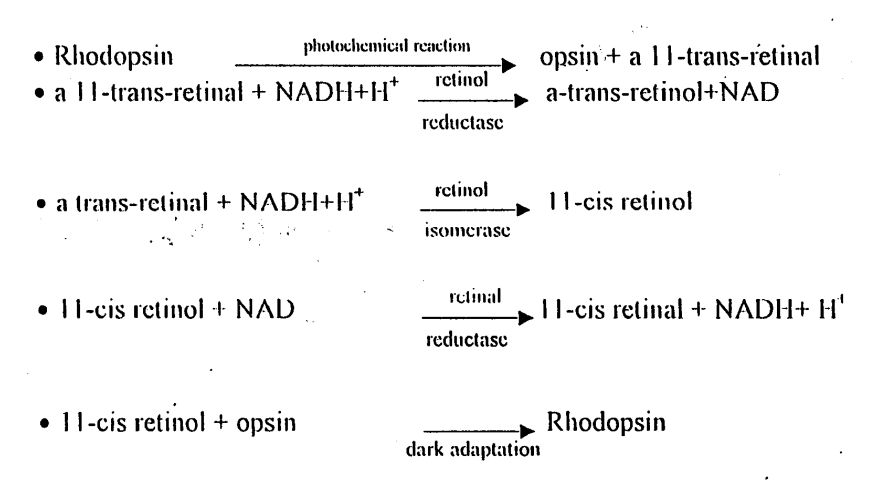
- ***Chemistry***:
  - Vitamin A (retinol) occures in two forms; Vit. A_1 is the common form in mamalian tissues and marine fishes is more potent than Vit. A_2.
  - The second is Vit. A2 (retinol 2) common in fresh water fishes, has double bond at $C_3 - C_4$, so is called 3-dehydroretinol (A_2), is less potent (40% that of Vit. A_1).
  - Vit. A does not occur in plants, but can be derived in human intestine from plant carotenes (pro Vit. A) by cleavage of β-carotene at the middle of its side chain giving rise to two moles of retinal (Vit. A-CHO.aldehyde).
  - The reaction is catalysed by β-carotene dioxygenase in presence of O_2 and bile salts.
  - Retinal is converted to retinol (Vit A-CH_2OH.alcohol) by reduction with retinal reductase and NADPH + H^+.
  - Small fraction of retinal is oxidized to retinoic acid in intestine, (Vit.A-COOH)
  - Vit A consists of six carbon cyclic ring (β-ionine ring) and eleven carbon side chain.
  - Vitamin-A is water insoluble, yellowish oily compound, thermostable, sensitive to oxygen and U-V.
  - About 95% of Vit. A is stored in liver as ester with pulmitic acid.
  - About 10-20mg of Vit. A is present per 100 gm of liver.

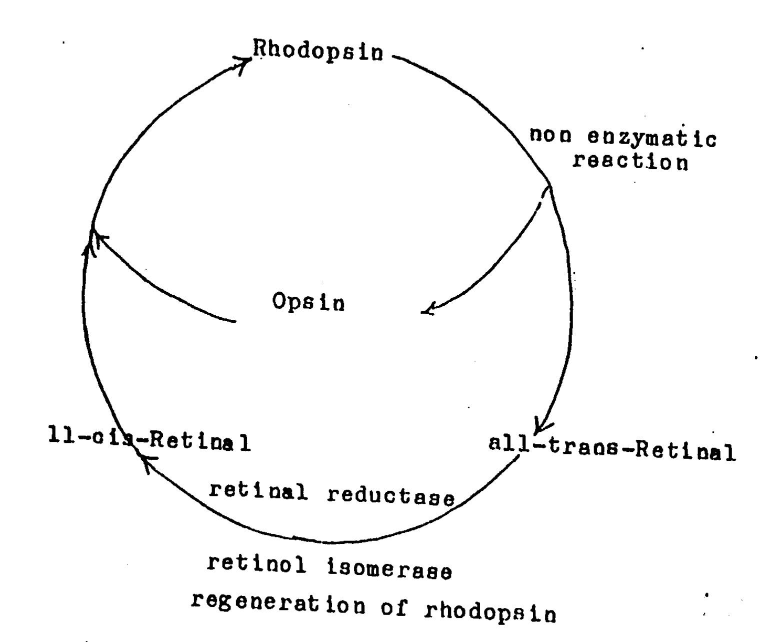
- ***Sources***:
  - Carotenes (pro. Vitamin A) are present in green leafy vegetables, apricots, cow's milk, carrots, and tomatoes.
  - Vitamin A is present in animal products, such as, cod-liver oil, halibut liver oil, shark liver oil, animal liver, kidney, butter, and egg yolk.

- ***Biochemical function of Vit. A*:**

  1- Retinal: has a significant function in vision at dim light (dark)

  - Retinal is aldehyde form derived from oxidation of retinol.
  - Retina contains two types of light sensitive receptors:
    - i) Rod cells for vision in dim light (dark)
    - ii) Cone cells for vision on bright light, and colour vision.
  - Rod cells contain compound protein called rhodopsin [consists of protein opsin, tightly bound to non part; 11-cis retinal aldehyde form of Vit. A)
  - When retina is exposed to light → photochemical reaction → rhodopsin is bleached → dissociates into opsin, and a 11-trans-retinal which triggers a nerve impulse which is transmitted by optic nerve to brain.
  - Then rhodopsin is regenerated from opsin and a 11-trans-retinol which undergoes isomerization back to a 11-cis retinal, this (visual cycle) is mainly enzymatic:



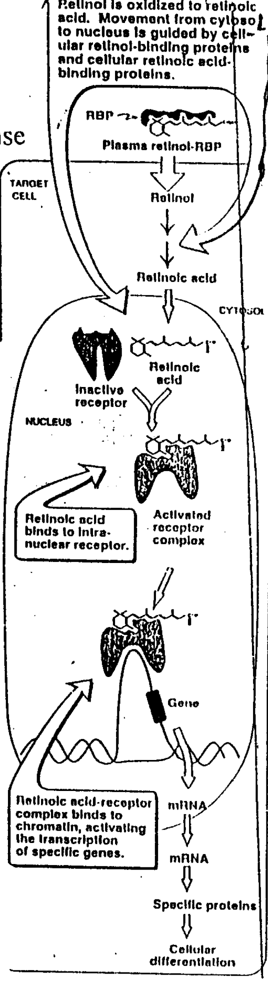


VISUAL CYCLE

2- ***Retinol***: is Vit. A containing primary alcohol group [-CH_2OH], retinol is found in animal tissues and liver as retinyl ester with long chain fatty acids. (retinyl palmitate).

- Retinol and retinal are essential for normal production, supporting spermatogenesis in male, and prevent fetal resorption in female.
- Retinol is released from liver to target cells, where it carried on plasma retinol-binding protein, then in the target cell retinol is oxidized to retinoic acid.
- Retinoic acid in nucleus binds to intranuclear receptor.
- Retinoic acid-receptor complex binds to the chromatin, and activates the transcription of specific genes → specific proteins → cellular differentiation for healthy epithelium, growth promotion, and

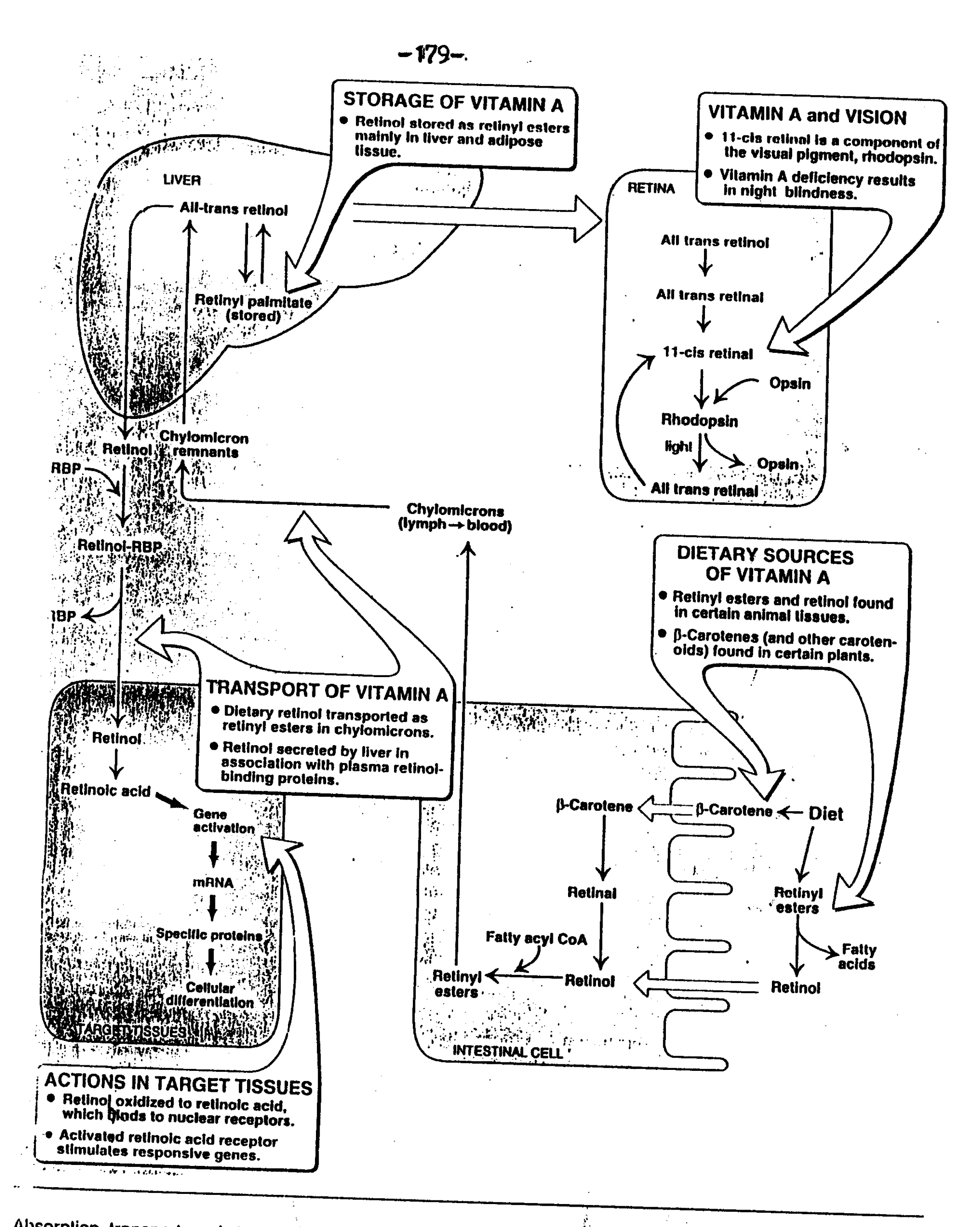
glycoprotein synthesis. Retinoic acid inhibits collagenase → prevent breakdown of collagen.



- ***Deficiency***:
  - Delay in dark adaptation, and loss of night vision (nyctalopia) are early signs.
  - Keratomalacia and xerophthalmia:

    Cornea and conjunctiva become dry, rough, corneal opacity, softness and corneal perforation may result, and end in loss of vision.
  - *Skin changes*:

    Dryness, roughness, papular eruption (goose skin) and follicular hyperkeratosis → crack → ulcer formation.
  - Urinary and respiratory tracts infections due to unhealthy endothelium.



Absorption, transport, and storage of vitamin A and its derivatives. RBP = retinol-binding protein.

- ***Clinical indications***:

  1) Night blindness, and xerophthalmia.

  2) Dermatologic disorders:

     Acne, psoriasis (treated by retinoic acid) and dry skin.

  3) Prevnction of chronic diseases:

  - β.carotene (provitamin A) decreases incidence of coronary heart diseases, lung and skin cancer.
  - β.carotene reduces risk of cataract and macular degeneration.
  - The protective action of β.carotene may due to its antioxidant properties, or to enhancement of body immune function.
  - β-carotene acts as anticancer by increasing MCH-1 receptors (major histocompatibility complex) which direct the killer T.cells or CD8 to kill cancerous foreign cells.

- ***Toxicity***:

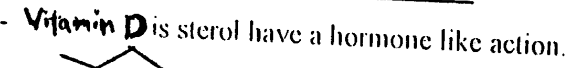
  - Hypervitaminosis-A may result from excessive intake of Vit. A if daily amounts exceeding 7.5 mg retinol/day.
  - Early signs are reflected in skin which becomes dry and pruritic.
  - Liver becomes enlarged and cirrhotic.
  - C.N.S manifestations due to increase in intracranial pressure.
  - Pregnant females may suffer form congenital malformations in fetus.
  - Unlike Vit. A, β.carotene is not toxic even at high doses.

- ***Normal serum value***:

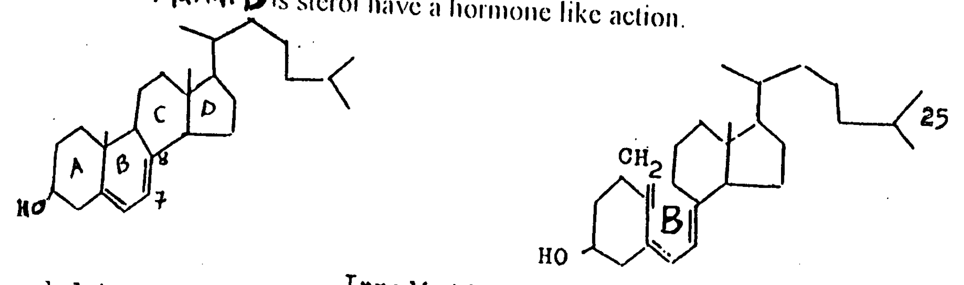
  - 30-80 μg/dl in adults
  - values above 100 μg/dl indicate toxicity
  - 0.3 μg of all-trans retinol or 0.6 μg of β.carotene is equal to(1)I.U.

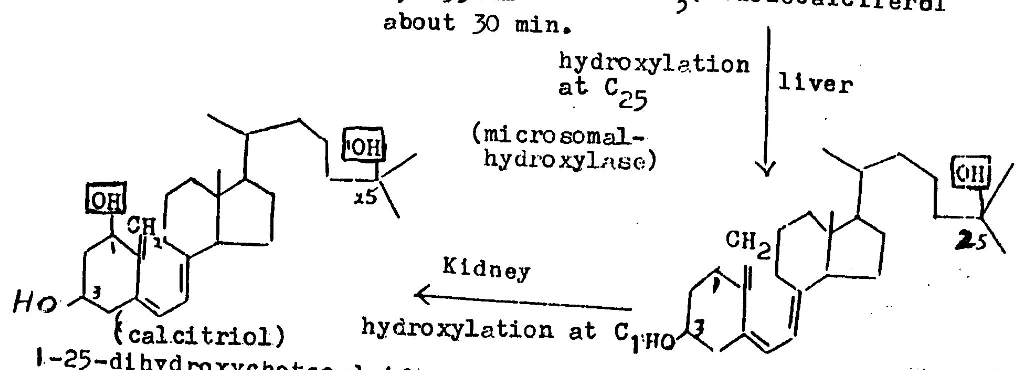
- *Normal daily intake*:
  - 800-1000 µg retinol /day (3000 – 5000 IU / day)
  - For β carotenes: one µg of retinol is equivalent to 6µg, β.carotene, or 12µg for α, or γ carotenes.

Vitamin D



- Vitamin D is sterol have a hormone like action.





- *Biochemical properties*:
  - Vitamin D is soluble in fats and fat solvents, thermostable, resists oxidation.
  - Ergosterol in plants is changed into vitamin D_2 (ergocalciferol) by U.V

$$\text{Ergosterol} \xrightarrow[\text{ultra violet rays}]{\text{U.V.}} \text{Ergocalciferol (Vit. } D_2)$$

- 7. Dehydrocholesterol under skin is changed by U.V. rays into cholecalciferol (Vit. D_3), where B ring is opened, and methyl group at C_{19} is changed into methylene group ($-CH_2$)

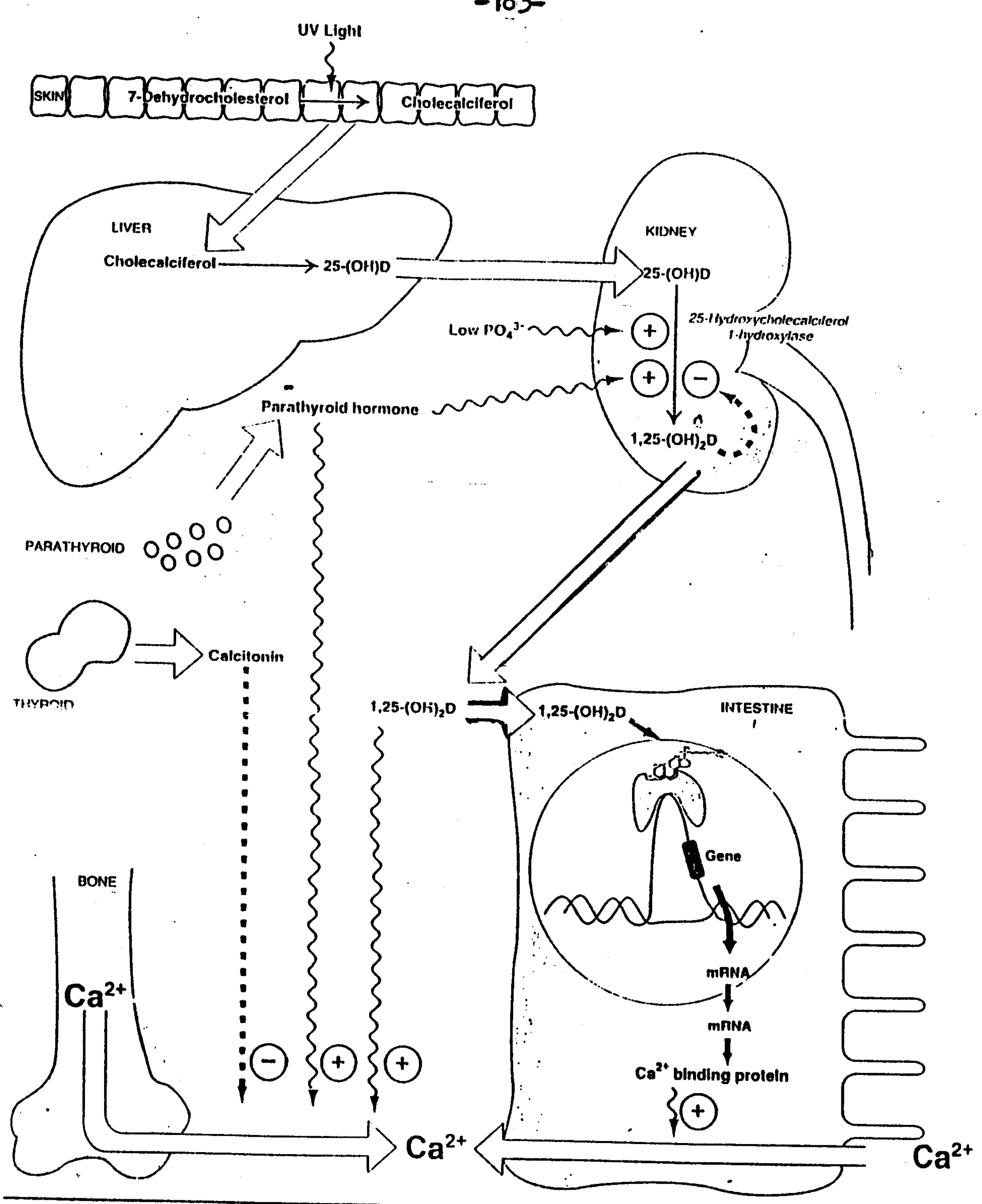
  7. Dehydrocholesterol $\xrightarrow[290-330nm]{U.VB}$ Cholecalciferol (Vit. D_3).

- Exposure to sunlight about ½ hour → synthesis of Vit. D for daily requirements (endogenous source)

■ ***Metabolism:***

Dietary Vit. D_2 or D_3 mixes with intestinal micelles, and bile salts, where they included with chylomicrones and abosrbed via lymphatics to liver.

- In liver cholecalciferol is hydroxylated at C_{25} by microsomal specific D_3-25 hydroxylase giving rise to 25. Cholecalciferol (25. OH.D_3), is the predominant form in plasma, and is major storage in body.
- 25. Cholecalciferol binds to α. globulin binding protein in plasma, and in kidneys a second hydroxylation occurs at C_1 via mitochondrial 1-hydroxylase with aid of [cyt.chrome P450, mol. Oxygen, and NADPH] to yield 1,25-dihydroxycholecalciferol (1, 25-di.OH.D_3); calcitriol.
- 1,25-di.OH.D_3; (calcitriol) is the most potent form of Vit. D.
- mitochondrial 1-hydroxylase in kidney is regulated by parathyroid hormone.
- In hypophosphatemia or hypocalcemia, there is stimulation of parathyroid hormone → stimulation of mitochondrial 1-hydroxylase → increase formation of plasma 1,25-di.OH.D_3 → increase plasma calcium.



Metabolism and actions of vitamin D.

■ *Biochemical function of active Vit. D*:

- The overall function of 1,25-di-OH.D_3 is to maintain adequate plasma calcium level through its effect on:

a) *Intestine*:

- 1,25-di-OH.D_3 stimulates intestinal absorption of calcium and phosphorus.
- It enters intestinal cells and binds to intracellular receptor protein, then the Vit. D-receptor complex moves to nucleus where it reacts with DNA, and produces specific mRNA for synthesis of a specific calcium-binding protein to carry more calcium to the blood, this mechanism is typical of steroid hormones.

b) *Bone*:

- 1, 25-di-OH.D_3 stimulates mobilization of calcium and phosphate from bone → increase plasma calcium and phosphate.
- In general, hypocalcemia stimulates parathyroid hormone, which in turn stimulates synthesis of 1,25-di-OH.D_3 → increase blood calcium.

c) *Kidney*:

- 1, 25-di.OH.D_3 decreases loss of calcium and phosphate from kidney to maintain blood calcium.

■ *Clinical indications of Vit. D*:

a) Rickets in children, which is characterized by demineralization of bone, in spite of continued collagen formation → soft pliable bones → delay teething, sitting, standing, walking and dental caries is common.

- Also, boxy skull, bow legs, and pigeon chest.

b) Osteomalacia, occurs in poor ignorant adult females due to multiple pregnancy, and bad dietary habits.

- There is bone deformities, low blood calcium and phosphorus.

c) Renal rickets, occurs in chronic renal failure → decrease formation of 1,25-di.OH.D_3 (calcitriol), so active calcitriol should be administrated.

d) Hypoparathyrodism:

- Lack of parathyroid hormone causes hypocalcemia and hyperphosphatemia.
- The patients may be treated with any form of Vit. D with parathyroid hormone.

■ *Toxicity*:

- Vit. D is the most toxic of all vitamins, because it stored in the body, and slowly metabolized.
- High doses (100.000 I.U) for weeks or months → loss appetite, nausea, thirst, and hypercalcemia → increase deposition of calcium in many organs, such as arteries, and kidney → renal stones.

■ *Normal serum values*:

- Calcitriol : 15-65 pg/ml
- 25-hydroxy. Vit. D: 15-60 ng/ml

■ *Daily requirements*:

- 400-800 IU/day (10-20 μg/ml)

Vitamin E (Tocopherols)

- Vitamin E consists of eight naturally vitamers.
- The most active is α-tocopherol.

• Vitamins E are derivatives of tocol nucleus.

HO 6, 5, 4, 3, 2, 1, 7, 8 CH_3 $(CH_2)_3$ - $\overset{CH_3}{CH}$ $-(CH_2)_3-$$\overset{CH_3}{CH}$ $-(CH_2)_3-$ $\overset{CH_3}{CH}$ CH_3

R

Tocol nucleus

α-Tocopherol

HO 6, CH_3 5, 4, 3, 2, CH_3, R, CH_3 7, 8 CH_3

VIT.E

- ***Chemistry***:
  - Tocopherols are derivatives of the tocol nucleus, in which α-tocopherol is trimethyl (5, 7, 8-trimethyl tocol)
  - Tocopherols are soluble in organic solvents, stable to acid and heat, but affected by oxygen and U.V.

- ***Metabolism***:
  - Vit. E is absorbed from intestine in presence of bile, and associated with chylomicrones, and stored in adipose tissues and phosopholipids of cell membranes.
  - Vit. E is oxidized in body and conjugated with glucuronic acid to be excreted in bile.

- ***Sources***:
  - Best sources are vegetable oils (wheat germ oil, cotton seed oils), also milk, and egg yolk.
  - Found in small amounts in lettuce, and peanut

- ***Biochemical function***:
  - Vit. E is an antioxidant, that acts as scavenger for molecular oxygen and free radicals, it prevents peroxidation on unsat. F.A. of cell membranes → maintains its normal integration.
  - Vit. E prevents oxidation of polyunsaturated fatty acids, thus preventing the toxic effects of fatty acid peroxides in tissues.
  - Vit. E prevents oxidation of LDL, because its oxidized form is atherogenic and promote heart disease (100 mg/day is required to decrease risk of heart diseases).
  - Vit. E protects RBCs from hemolysis by decreasing effect of H_2O_2 on cell membrane.
  - Vit. E provides some benefit in atherosclerotic peripheral vascular disease by inhibition of lipid peroxides, platelets aggregation, and elevation of HDL.
  - Supplements of Vit. E protects against atmospheric pollution , such as ozone and nitrous oxide.
  - Vit. E may be used in nocturnal muscle cramp, fibrocystic breast disease, and intermittent claudication.
- ***Toxicity of Vit. E***:
  - Vit. E is the least toxic fat soluble vitamin, no toxicity has been observed even at 300 mg/day.

- ***Normal serum values***:
  - 0.5-1.8mg/dl in adults (is carried mainly on α lipoprotein fraction)
  - 0.1-0.5 mg/dl in prematures

- ***Requirements***:
  - Need of Vit. E is increased in relation to amount of polyunsaturated fatty acids in diet.

- Adult range: 25-35 mg/day
- 1mg α tocopherol is equal to 1.49 I.U.

Vitamin K

Vitamin K promotes blood clotting by conversion of prothrombin (F.II), and factors VII (proconvertin), IX (plasma thromboplastin), X (Stuart factor) to active clotting factors.

. 2 , methyl - 1,4 naphthoquinone ring .

$CH_2-CH=\overset{CH_3}{\overset{|}{C}}-CH_2-(CH_2-CH_2-\overset{CH_3}{\overset{|}{CH}}-CH_2)_3H$

Vitamin K_1 (Phytyl radical) R

$(CH_2-CH=\overset{CH_3}{\overset{|}{C}}-CH_2)_nH$ (n = may be 6,7,8,9 ,10 depending on the species).

Vitamin K_2 (difarnesyl Radical) R

CH_3 Vitamin K_3

- *Chemistry*:
  - There are 3 types of vitamin K:
  1) Vitamin K_1 (phylloquinone): synthesized in green leafy plants, cabbage, and spinach. It possesses a phytyl side chain on position 3.

Menaquinone

2) Vitamin K_2 (farnoquinone): occurs in bacteria has a difarnesyl side chain, is important source of vitamin K in the body.

3) Vitamin K_3: is synthetic methyl (menadione structure, water soluble) has no long side chain, is more potent 3 times more than natural forms.

- Vitamin K is solvent in organic fat solvents, destroyed by alkaline solution, reducing agents, and U.V light.
- Vit. K_1, K_2 are absorbed with aid of bile, bound to chylomicrones → stored in liver, then distributed to other tissues with association of LDL.

- *Biochemical function of vitamin K*:
  - Activation of blood clotting factors, such as prothrombin, and factors (VII, IX, X) through carboxylation of their glutamate residues into γ-carboxy glutamic acid which contains two negative charge carboxylic groups ($2COO^-$)

γ COO^- / β CH_2 / α CH_2 / $-C(=O)-CH-NH-$ —(CO_2, vitamin K)→ ^-OOC COO^- / CH / CH_2 / $-C(=O)-CH-NH-$ —(Phospholipid, Ca^{++})→ Ca^{++} chelated by two COO^- / CH / CH_2 / $-C-CH-NH-$

glutamate γ-carboxy glutamate protein-Ca^{++} chelate

- These groups act as chelating of positive charge calcium ions (Ca^{++}) to form active prothrombin – calcium complex which binds to phospholipid layer on platelets cell membrane and converts prothrombin into active thrombin.

- Vitamin K may act as an cofactor for respiratory chain oxidation in mitochondria (acts as electron carrier), it has benzoquinone ring, and called coenzyme Q or ubioquinone.

O

CH_3-O — CH_3 (isopernaid side chain)

CH_3O — ----- (R)

O

COENZYME Q (UBIQUINONE)

- Osteocalin protein (bone binding protein) contains γ-carboxy glutamic acid, in which vitamin K take part in its formation.

■ *Deficiency occurs in*:

- Newly born infants (due to sterility of large intestine specially in first week)
- Also management of severe diarrhea by intestinal antiseptic → hypoprothrombinemia due to inhibition of vitamin K_2, so supplementatin of vitamin K is required.
- Second generation of systemic antibiotic cephalosporins, such as cefoperazone, cefamandole may cause hypoprothrombinemia.
- In liver diseases, prothrombin formation is inhibited.
- In obstructive jaundice vitamin K is not absorbed due to deficiency of bile salts.

■ *Toxicity*:

- Large doses of vitamin K can produce hemolytic anemia and jaundice in infants due to toxic effects on red blood cells membrane.

- ***Normal serum value:***
  - 13 –120 ng/dl

- ***Daily requirements:***
  - 70-140 μg/day

Study Questions: Choose the ONE best answer.

Questions 28.1 to 28.6

For each description (below) select the most appropriate vitamin.

28.1 Required in oxidative decarboxylation

28.2 Contains a glutamate residue

28.3 Participates in the conversion of homocysteine to methionine

28.4 A water-soluble antioxidant

28.5 High doses are contraindicated in pregnancy

28.6 Precursor of a vitamin

A. Biotin
B. Vitamin B_{12}
C. Ascorbic acid
D. Thiamine
E. Riboflavin
F. Folic acid
G. Vitamin A
H. Vitamin D
I. Vitamin K
J. Vitamin E
K. β-Carotene

> Correct answers
> 28.1 D: Thiamine
> 28.2 F: Folic acid
> 28.3 B: Vitamin B_{12}
> 28.4 C: Ascorbic acid
> 28.5 G: Vitamin A
> 28.6 K: β-Carotene

28.7 Which one of the following statements concerning vitamin B_{12} is INCORRECT?

A. It can be converted to cofactor forms containing a 5'-deoxyadenosine or methyl group attached to cobalt.

B. It can serve as a source of a cofactor required for the conversion of methylmalonyl CoA to succinyl CoA.

C. It requires a specific glycoprotein for its absorption.

D. It may be present in inadequate quantities in a strictly vegetarian diet.

E. It contains a heme group.

> Correct choice = E. Vitamin B_{12} contains a corrin ring not a heme group.

28.8 Which one of the following statements concerning ascorbic acid is INCORRECT?

A. Dehydroascorbate is a nontoxic metabolite of vitamin C.

B. It is a cofactor required for the hydroxylation of proline and lysine.

C. It is an antioxidant.

D. Diets high in ascorbic acid reduce the risk for certain types of cancer.

E. Diets deficient in ascorbic acid can lead to a syndrome called scurvy.

> Correct choice = A. Dehydroascorbate is a toxic intermediate of ascorbate metabolism that could potentially accumulate in some individuals who routinely consume very high doses of the vitamin.

28.9 Which one of the following statements concerning niacin is correct?

A. Niacin is the coenzyme for carboxylation reactions.

B. Niacin is the precursor of FAD.

C. Niacin-rich foods are effective in treating hyperlipidemia.

D. The nutritional requirement for niacin in humans is decreased when the diet contains large amounts of animal protein.

E. Niacin deficiency leads to beri-beri.

> Correct answer = D. Animal proteins are a good source of tryptophan, which can be metabolized in part, to niacin. Biotin is the coenzyme for carboxylations. Riboflavin is the precursor of FAD. Only high supplemental doses of niacin are effective in treating hyperlipidemia. Niacin deficiency leads to pellagra.

28.10 A deficiency of biotin in a higher animal is likely to be accompanied by:

A. defective oxidation of fatty acids to acetyl CoA.

B. decreased formation of lactate in skeletal muscular contraction.

C. decreased oxidation of succinate by mitochondria isolated from the liver of biotin-deficient animals.

D. defective synthesis of fatty acids.

E. night blindness.

> Correct answer = D. Biotin is required for the carboxylation of acetyl CoA to malonyl CoA, an intermediate in fatty acid synthesis.

Retinol:

A. can be enzymically formed from retinoic acid.

B. is transported from the intestine to the liver in chylomicrons.

C. Is the light-absorbing portion of rhodopsin.

D. is phosphorylated and dephosphorylated during the visual cycle.

E. mediates most of the actions of the retinoids.

Correct answer = B. Retinyl esters are incorporated into chylomicrons. Retinoic acid cannot be reduced to retinol. Retinal, the aldehyde form of retinol, is the chromophore for rhodopsin. Retinal is photoisomerized during the visual cycle. Retinoic acid, not retinol, is the most important retinoid.

28.12 Which one of the following statements concerning vitamin D is correct?

A. Chronic renal failure requires administration of 1,25-dihydroxycholecalciferol.

B. It is required in the diet of individuals exposed to sunlight.

C. 25-Hydroxycholecalciferol is the active form of the vitamin.

D. Vitamin D opposes the effect of parathyroid hormone.

E. A deficiency in vitamin D results in an increased secretion of calcitonin.

Correct answer = A. Renal failure results in the decreased ability to form the active form of the vitamin, which must be supplied. The vitamin is not required in individuals exposed to sunlight. 1,25-dihydroxycholecalciferol is the active form of the vitamin. Vitamin D and parathyroid hormone both increase serum calcium. A deficiency of vitamin D decreases the secretion of calcitonin.

28.13 Vitamin K:

A. plays an essential role in preventing thrombosis.

B. increases the coagulation time in newborn infants with hemorrhagic disease.

C. is present in high concentration in cow's or breast milk.

D. is synthesized by intestinal bacteria.

E. is a water-soluble vitamin.

Correct answer = D. Vitamin K is essential for clot formation, decreases coagulation time, and is present in low concentrations in milk.

28.14 Which of the following statements about fat-soluble vitamins is INCORRECT?

A. Diets rich in vitamin E decrease the risk for coronary artery disease in adults.

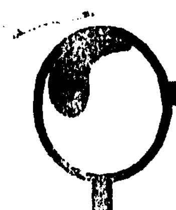
B. Vitamin E deficiency is almost entirely restricted to premature infants.

C. Certain second generation cephalosporins, such as cefoperazone, cefamandole, and moxalactam can cause hypoprothrombinemia.

D. Vitamin K therapy is often required during the first year of life.

E. α-Tocopherol requirements increase with the amount of polyunsaturated fatty acid in the diet.

Correct choice = D. Newborns typically receive a single intramuscular dose of vitamin K as prophylaxis against hemorrhagic disease. Vitamin E protects against heart disease. The antibiotics can antagonize the action of vitamin K. α-Tocopherol requirements increase with the amount of polyunsaturated fatty acid in the diet.



Xenobiotics Metabolism

DEFINITION:

- Xenobiotics are chemical and organic substances toxic (foreign) to body, may be precancerous, such as environmental pollutions, food additives, drugs, and insecticides.
- There are about 200.000 types in nature.
- Xenobiotics if not metabolized properly in body, it may cause cell destruction or cancer.
- Liver is the main organ involved with xenobiotics metabolism, also vital organs such as kidney and lung.

SOME TYPES OF ENVIRONMENTAL POLLUTIONS:

1) Food additives:

a) Artificial colours and flavours.

b) Aflatoxin B of beanut fungus (potent hepatocarcinogenic)

c) Hydrocarbones as in phenanthrene nucleus (methyl cholanthrene) in prolonged heated oils (↑ viscosity, change in colour)

2) *Synthetic drugs*:

- Drugs in abuse, some antibiotics depress immune system, addictive drugs and some synthetic drugs.

  So, drugs of natural origin is better.

3) *Drink pollution*:

- Water pollution with bacteria (salmonella) or sewage.
- Water pollution with heavy metals (lead, lithium, mercury) from factory disposal. It has dangerous accumulative effects which affect liver, kidney, bones, and C.N.S. (lead causes mental retardation).

*Fat soluble drugs, has accumulative effects, so they are considered as xenobiotics.

4) *Air pollution*:

- Inhalation of volatile chemical reagents as chloroform, pyridine, volatile glue, and insecticides.
- Negative smoking (aromatic hydrocarbones), cars pollution (lead).



5) ***Skin and dressing pollution***:

- AS cosmotics (powder or cream), tattoing (toxic pigment), synthetic dressing may cause allergy.
- X. Ray, and U.V; may cause cell mutation and cancer in nucleotides causing thymine dimer (in one strand of DNA) at wave length about 260nm, this may be corrected by DNA repair.
- 50% of scandivean whose immigrate to Australia may affected with melanoma.

6) ***Noise trauma***:

Causes sensory hearing loss [sounds exceeding 58dB]. causes of noise may be industrial machines, loud music, and weapons. Earplugs should be used when exposed to loud noises.

7) ***Electromagnetic waves***: [high +ve charges]

From high voltage electric station, T.V., mobile, may cause cancer, sterility, and hyperlipedemia.

** 80% of human cancer is environmental POLLUTION.



MECHANISM OF XENOBIOTICS METABOLISM

- Xenobiotics are metabolized in body to be easily excreted with aid of two enzyme systems:-

  a) Hydroxylation through the mono-oxygenases (cytochrome P450) to render the xenobiotics more soluble, less toxic, easily excreted.

  - Cytochrome.P450 (mono.oxygenase) is hemoprotein enzyme containing Fe^{++} (ferrous) ion.

  b) Conjugation and excretion through the transferases enzymes.

STEPS OF XENOBIOTIC METABOLISM:

Is divided into two phases:

- **Phase I: (Hydroxylation)**

  Is hydroxylation of xenobiotics by introducing one atom of oxygen to it via the cytochrome p450 forming less toxic and more soluble reactive compound for easily excretion in urine or bile.

  - *Requirements of the reaction*:

  The reaction is catabolized by:

1) Reduced cyt. P450 enzyme   2) molecular eoxygen

3) Reduced NADPH + H^+ 4) The xenobiotics

Example:

i- R-H $+O_2$ (toxic xenobiotic) $\xrightarrow[NADPH+H^+]{\text{Reduced cyt. p450}}$ R-OH (Reactive less toxic metabolite) $+ H_2O +$ NADP + oxid.cyt. P450. (Fe^{+++})

or (R.H +O2 $\xrightarrow[NADPH+H]{\text{Red. cyt. 450}}$ cyt.p450 Fe+++ R.OH +NADP +H2O).

- Cyto.P450 can split O_2, and binds one [O] atom to its ferrous iron (Fe^{++}) → Fe^{+++}.O [ferric oxide]
- Then Fe^{+++}.O + R-H → R-OH + Fe^{+++}
- The other oxygen atom is reduced to H_2O by NADPH+H^+

ii-Oxidized cyt. p450 (Fe^{+++}) $\xrightarrow[NADPH+H]{\text{reductase cyt. P450}}$ reduced cyt. p450 (Fe^{++}) for further use.

or (cyt.P450-Fe^{+++} $\xrightarrow[NADPH+H]{\text{cyt.450 reductase}}$ cyt.P450—Fe^{++}).

** In some cases the xenobiotic may bind firmly to cell macro organills [DNA, RNA, cell protein] producing cell injury or mutation [changed into more toxic active biologically compound], so in this case further

hydroxylation is required by the cyt. Chrome p450 system.

.OTHER FUNCTIONS OF CYTO.CHROME.P 450:-

- Synthesis of steroids.
- Synthesis of eicosanoids(20 C faty acids;leukotrienes).
- Activation of vit.D3.

☐ Phase II: (Conjugation & Excretion):

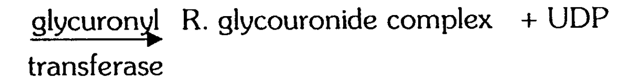
- The reactive metabolite in phase I is conjugated with different polar active substances to be more soluble and easily excreted from body.
- The reaction is catabolized by transferases enzymes.
- *Types of conjugation reactions*:

1) Glucuronidation

- Is the most frequent reaction by the active UDP Glucuronic acid and glucuronyl transferase present in endoplasmic reticulum (E.R) of microsomes of liver cell. (the same site of cyt. p450).
- This reaction detoxify carcinogens, aniline, benzoic acids, phenols, and many steroids are excreted as glucuronides in bile or urine

Example:

R-OH(reactive metabolite +UDP glucuronic acid



glycuronyl → R. glycouronide complex + UDP
transferase

2) Sulfation

By active sulphate (PAPS): ph. Adenosine.ph. SO_4) for detoxification of alcohols, phenols, steroids, and galactolipids by using active sulphate as (phosphoadenosine phosphosulfates)

R-OH+PAPS $\xrightarrow[\text{transferase}]{\text{PAPS}}$ R-sulphate complex → urine

(AR_P~P~P + SO_4 $\xrightarrow{-Pi}_{+SO_4}$ AR_P~ P_SO_4)
ATP PAPS

3) Conjugation with glutathione

- Glutathione is tripeptile (glut, cyst (SH), glycine) present in active reduced state (G.SH).
- Some carcinogen xenobiotics are conjugated to G.SH to render the xenobiotic less toxic and easily excreted, through glutathione transferase enzyme.

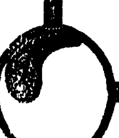
Example:

R.OH+2G.SH $\xrightarrow{\text{glutathione transerase}}$ R.2G.S.+NADPH+H^+ (= R<GS GS)

Reduced glutathione; NADP → NADPH+H

↓

(Non toxic oxid. glutathione-xenobiotic complex)



- If carcinogen xenobiotics not conjugated to G.SH would be free to combine with cell macro organills leading to serious cell damage or mutation.
- Therefore G.SH is defense mechanism against certain toxic compounds such as some drugs and carcinogens.

4) Acetylation :

- By conjugation with acetyl CoA [CH_3.Co.ScoA] for some drugs as isoniazid (T.B drug)
- Enzyme catabolize that reaction is acetyl transferase which is present in many forms (isozymes) for fast and slow acetylation (clearance of the drug);
- The drug has toxic accumulating effect.

R-OH+acetyl CoA $\xrightarrow[\text{Transferase}]{\text{acetyl}}$ acetyl-R complex + CoASH
↓
bile or urine

or:

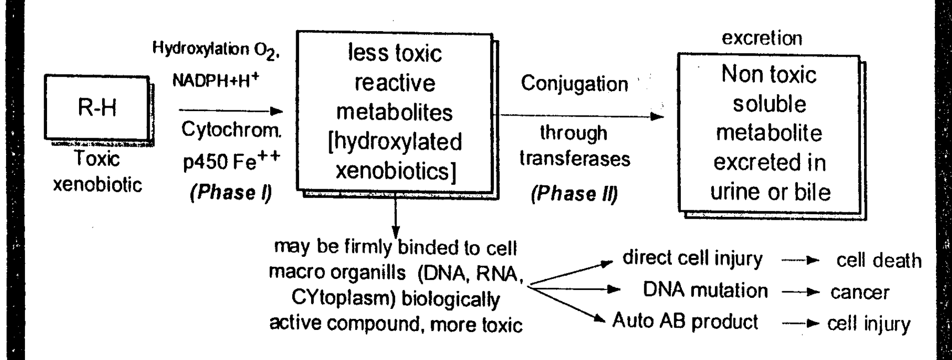
[R.OH+CH_3.CO.ScoA $\xrightarrow{\text{A.Transferase}}$ CH_3.COO.R+CoASH]

5) Methylation: (conjugation with $-CH_3$ group)

- Few xenobiotics are subjected to methylation using active adenosyl methionine [methyl donor]
- The reaction is catabolized by the methyl transferase.

$$\underset{\underset{\text{(active methionine)}}{\text{A.R}\sim\text{S.}CH_3}}{CH_2}.CH_2.\underset{NH_2}{CH}.COOH + R.OH \xrightarrow[\text{transferase}]{\text{methyl}} \text{methyl.xenobiotic acceptor } (R\text{-}CH_3)$$

SUMMARY OF XENOBIOTIC METABOLISM:



FACTORS AFFECTING XENOBIOTIC METABOLISM

1) Species:

Activity of xenobiotic metabolizing enzymes differ from one species to others or toxicity differs from one species to others (black races are more affected with T.B. than whites).

2) ***Genetic factors***: (Genetic disposition)

Enzyme activity differs widely according to inherited genetic factors.

3) ***Age & Sex***:

Enzyme activity varies according to age and sex, extreme of age → ↓ immunity → cancers

4) ***Enzyme induction***:

Some drugs as phenobarbitone can induce (stimulate) cytochrome p450 enzymes increasing its concentration.

5) ***Some chemicals***: as cyanides can inhibit the cytochrome enzymes even at low levels.

6) Obesty may lead to cancer, because fat soluble carcinogenic substances are stored in fat tissuses.

<u>THE EPOXIDES</u> (toxic xenobiotic) and epoxide hydrolase:

- **Epoxides**:

Are toxic products due to action of certain cytochrome p450 on some procarcinogens (as hydrocarbones) which are more toxic and highly active carcinogen.

- **The epoxide hydrolase**: (is analogue to cyt.p450):
  - Present in endoplasmic reticulum (ER) of the cell, has protective effects against certain epoxides [carcinogens]
  - It converts the toxic epoxide into much less toxic dihydrodiols (by hydroxylation)

R(CH) —Cyt.p450 / O_2, $NADP_2H$→ H–C–C–H (epoxide, bridged by O) —epoxide hydrolase / H_2O→ H-C - C-H (with OH OH)

Active toxic epoxide

less toxic dihydrodiols
(dihydroxylated → more soluble)

CHEMISTRY OF CYTOCHROMES P. 450

- Are iron hemoprotein enzymes present in active reduced form [Fe^{++}] in endoplasmic reticulum [ER] of the cells.
- There is microsomal type of cytochrome p450, present in liver, lung, kidney and brain for metabolism of fat soluble drugs, other xenobiotics (toxic, precarcinogenic compounds)
- **Mitochondrial type**: is present in adrenals (its protein has no iron) and has adrenodoxin reductase, plays role in biosynthesis of steroids from cholesterol, and catalyze excess of steroids.



- They are absorbed with high peak at wave length 450 nm.
- They act as electron carrier

Oxid. Cyto.chtrom.p450. Fe^{+++} $\xrightarrow[\text{NADP2H} \curvearrowright \text{NADP}]{+\bar{e} \text{ cytochrom. Reductase}}$ reduced cyto chrome (Fe^{++})

- Microsomal cytochrome.p450 in liver, is present at least in 6 species (sub types), its genes has been identified and isolated, more than 38 genes in human. Each one has wide specificity to various numbers of drugs and-carcinogens to combat (catabolize) them.
- The one subtype of cytochrome p450 may present in polymorphic (isozymes) form; some of which show low activity and others has fast activity, which explains variations in the drug response among many patients.
- Most species of microsomal cytochrome p450 in liver are inducible; administration of phenobarbitone causes increase 3-4 times of cytochrome p450 in 4-5 days due to stimulation of mRNA transcription of cytochrome p450.
- This elevation of cytochrome p450, may inhance inhibition of other drugs (anticoagulant) taken in this time (inhibitory drug interaction)

Phenobarbitone + anticoagulant $\xrightarrow[\text{cyto.chrome p450}]{\text{Induction of}}$ rapid detoxication of anticoagulant → ↓ its desired action [inhibitory drug interaction]

CHEMISTRY OF CYTOCHROME P448: (Aromatic hydrocarbon hydroxylase)

- Cytochrome p448 has high abosrption peak at wave length 448nm.
- Is other species of cytochrome p450 which is concerned with metabolism of pre carcinogens as poly cyclic aromatic hydrocarbones (xenobiotic) (PAHs) which is inhaled by smoking through the aromatic hydrocarbon hydroxylase (AHH) which is present in cytochrome p448 system via the hydroxylation reaction.
- Therefore, cytochrome p448 is increased in smokers.
- Cytochrome p448 may be elevated or induced in placenta of smoked women → Foetus deformity.

Xenobiotic (PAHs) in smoking $\xrightarrow[\text{NADPH+}O_2]{\text{Cyto.chrome p448}}$
- ↗ Hydroxylated product → conjugation
- → Firm binding to lung cells (active carcinogen) → DNA DAMAGE.

According to species, age, genetics



DIGESTION & ABSORPTION

DEFINITION:

Digestion is breakdown of foodstuffs into simplest (small) form to be easily absorbed by the aid of G.I.T. hydrolases enzymes.

- Carbohydrates (starch, glycogen, sugars) into monosaccharides
- Lipids (oils & fats) into fatty acids & monoacylglycerols.
- Proteins (animal & plant protein) into amino acids.

(I) Carbohydrates

- Constitute about 60% of human diet, which include plant starch, animal glycogen (liver & muscles), sucrose, lactose (milk & cheese), cellulose, fructose & glucose (fruits).
- The aim of digestion and metabolism of CHO elements is to extract its solar energy during its formation in plants by photosynthesis [$6CO_2 + 6\ H_2O$+solar energy) → $C_6H_{12}O_6 + 6O_2$
- In body carbohydrates are digested and oxidized to gain energy

$C_6H_{12}O_6 \xrightarrow{\text{metabolism}} CO_2+H_2O$ + energy for body activities

STEPS:

- *In mouth* (oral cavity):
  - Salivary amylase acts on starch, glycogen to yield maltose [starch →amylo.dext → erythro.dext.→ achro





dext. → maltose], according to time of mastication, the process is limited.

- Salivary amylase is inactivated in stomach at pH4.
- pH of salivary amylase 6.6-6.8, Cl^- ion is required.

- *In stomach*: no enzymes of CHO digestion.
- *In small intestine*:
  - *Deudinum*: (pancreatic amylase; pH 7.1): activated by Cl^- ion.
    * Is strong hydrolytic enzyme catabolize starch and glycogen into maltose (disaccharide).
  - *Jejunum and ileum*: pH (5-7):

Contain; maltase(pH 5.8-6.2): maltose $\xrightarrow{\text{maltase}}$ 2 glucose

Sucrase (pH 5-7): sucrose $\xrightarrow{\text{sucrase}}$ glucose + fructose

Lactase (pH 5.4-6): lactose $\xrightarrow{\text{lactase}}$ glucose + galactose

Inherited sucrase and lactase deficiency lead to abdominal colic, diarrhea, flatulence (gases) in childhood due to increase disaccharides in intestine, retaining water → diarrhea, and action of bacteria → gases and colic.

<u>*Cellulose*: (poly saccharaides) 1-4 β glucose:</u>

Not digested, no cellulase, ↑ bulk of stool → stimulates intestinal movement → evacuation (prevent constipation).

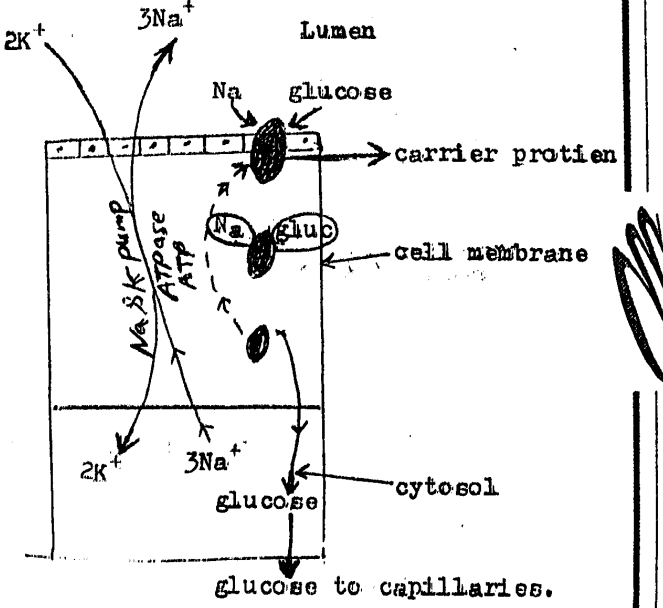


So, end products: of CHO digestion are glucose, fructose, and galactose whose are taken by the liver, where galactose and fructose are converted to glucose to be utilized in the body.

ABSORPTION OF MONOSACCHARIDE:

Monosaccharides can be absorbed by passive or active transport.

1) Passive transport: (sugars pass from high to low concentration, needs no energy, as pentoses, fructose.
2) Active transport: sugars pass from low to high concentration, it needs energy as glucose & galactose.
   In all membrane there is a mobile carrier which binds both glucose and Na^+, then they separate in cytosol.
   The carrier gets back to cell membrane, and Na^+ is pumped again to cell membrane by aid of ATP to carry glucose and once more.



ACTIVE GLUCOSE TRANSPORT*





ABSORPTION RATE:

Glucose (100), galactose (110), fructose (40), pentose (20)

(II) Proteins

Constitute about 15% of human diet, consist of long chains of polypeptides.

In oral cavity:

No digestion (no proteolytic enzymes)

In stomach:

Proteins in stomach stimulate gastrin hormone from lower third of stomach to stimulate HCl secretion.

- Gastrin stimulates HCl secretion from parietal cells (middle third of stomach)
- Hypoglycemia, hypercalcemia stimulate gastrin release.
- Gastric juice: contains: 0.2-0.5% HCl (pH about 1), pepsin, and rennin for protein digestion.

  1) *HCl*: protein diet stimulates secretion of gastric HCl from the parietal cells, which denatures the tertiary structure of proteins (become unfolded) for action of proteolytic enzymes.



→ OMEPRAZOLE (anti peptic ulcer drug) decrease secretion of HCL by inhibition of ATPase proton-K^+ pump....look figure.

Protein diet $\xrightarrow{(+)\ \text{gastrin}}$ stimulate HCl $\xrightarrow{(+)}$ pepsinogen → active pepsin



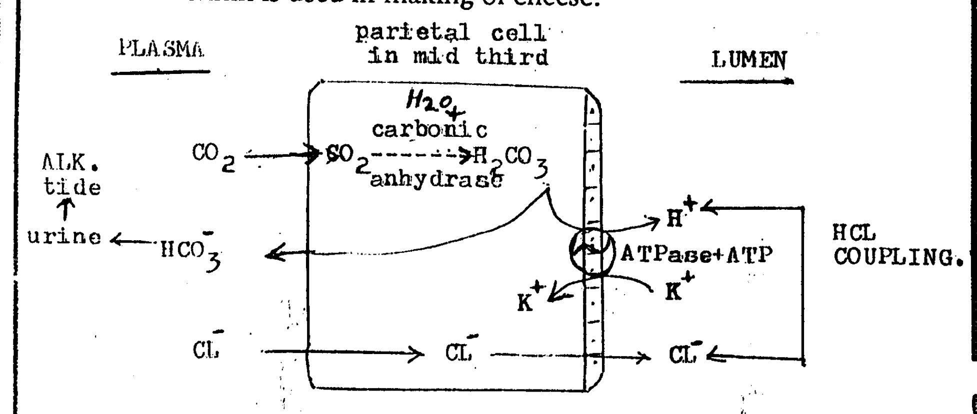


2) *Pepsin*:

Pepsin is present in inactive form (pepsinogen) which is stimulated by HCl to active pepsin, then active pepsin stimulates the inactive pepsinogen into active pepsin (auto activation).

Pepsin acts on peptide bonds within the main polypeptide chain (endopeptidase), yielding peptide chains from proteins.

3) *Rennin*: (pH4): acts on casein of milk, and cheese to produce paracasein, then with aid of Ca^{++} to form insoluble calcium caseinate (milk clot), preventing rapid passage of milk from stomach to intestine (give sense of fullness) in children. Rennin is used in making of cheese.



*HCO_3^- passes into plasma in exchange for CL^-
*H^+ passes to lumen in exchange for K^+ with aid of ATP, ATPase (proton & K^+ pump).

– ACTIVE SECRETION OF HCL –

In small intestine:

- *Duodenum*: by pancreatic secretion





- Presence of acid chyme from stomach to duodenum stimulates it to secrete secretin and hole cholecystokinin hormones to produce the pancreatic enzymes: Trypsin, chymotrypsin and elastase (as endopeptidases), in addition to carboxypeptidase (as exopeptidase).
- All mentioned (above) enzymes are present in inactive form.
- Trypsinogen is attacked by an intestinal enzyme (enterokinase), converting it into <u>active trypsin</u>.
- <u>Active trypsin</u> in turn converts trypsinogen → trypsin
  and chymotrypsinogen → chymotrypsin
  and proelastase → elastase
  Finally procarboxypeptidase → carboxypeptidase [acts on amino acid adjacent to carboxyl side of polypeptide chain]

- <u>End products</u>: short peptide chains are produced by (endopeptidases), and single free amino acid by (exopeptidase).

- *Jejunum & Ileum*: by intestinal secretion:

Digestion of short peptides are completed by aminopeptidase [is exopeptidase acts on amino acid adjacent N. terminal side] and dipeptidase which acts on dipeptides producing free amino acids.

<u>*Absorption:*</u>

- Free L. amino acids are transported to portal vein by active transport.
- Passive transport is to D. amino acids (not in human).





INTESTINAL PUTREFACTION AND FERMENTATION

- Each day the duodenum receives about 7-10L of ingested food, water, salivary gland secretions.
- Jejunum and ileum (20 feet), absorb maximum of water and nutrients, so that about 1.5 liters only reach the first part of large intestine (cecum)
- In large intestine (5 feet) more absorption of water takes place,, so that only 100-300 ml of semisolid stool is remained.
- Intestinal bacteria act on the solid material producing fermentation and putrefaction.
- Intestinal flora may form 25% of dry weight of stool, vit. k, some vit. B (niacin B_{12}) may formed by intestinal flora.

Putrefaction:

- Is decomposition of proteins and amino acids by intestinal anaerobic organisms producing offensive odours:

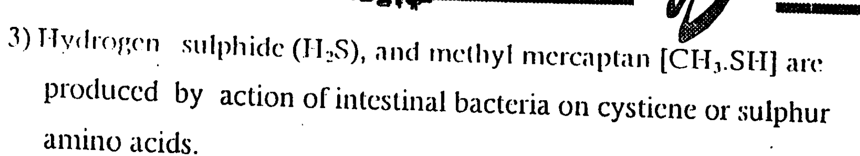
1) By decarboxylation of amino acids, toxic amines (ptomains; $R.CH_2.NH_2$) may produced (if in fish, meat,----> cause food poisoning)---> $R.CH(NH_2).COOH \xrightarrow[\text{decarboxylase}]{\text{bacterial}} R.CH_2.NH_2 + CO_2$.

   Tryptophan $\xrightarrow{-CO_2}$ tryptamine (vasoconstrictor)

   Histidine $\xrightarrow{-CO_2}$ Histamine (vasodilator)

2) Tryptophan undergo a series of reactions producing skatole and indole whose give the odour of stool.





3) Hydrogen sulphide (H_2S), and methyl mercaptan [$CH_3.SH$] are produced by action of intestinal bacteria on cysticne or sulphur amino acids.

4) By deamination of amino acids, ammonia is produced (toxic), in liver it changed into neutral urea, to be excreted in urine.

- In liver diseases, ammonia accumulates and cause hepatic coma.
- Production of intestinal ammonia is inhibited by neomycin.

Fermentation:

- Is decomposition of carbohydrates by intestinal bacteria
- CO_2, lactic acid are produced, which are non toxic as compare with putrefaction.

Examples:

1) E. coli on glucose → lactic acid, acetate, CO_2, H_2.

$$C_6H_{12}O_6 + H_2O \xrightarrow{E.coli} CH_3.CHOH.COOH + CH_3COOH + CO_2 + 2H_2$$

2) Fermentation by Bakers yeast (has zymases) of glucose, fructose produce ethanol + CO_2 + E (alcoholic fermentation)

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow[\text{Ferment.}]{\text{yeast}} \underset{\text{ethanol}}{2C_2H_5OH} + 2CO_2 + E$$

3) Also lactobacillus acidophilus acts on lactose → lactic acid.

$C_{12}H_{22}O_{11}$ lact. Bacillus $4CH_3.CHOH.COOH$ [lactate causes souring of milk and casein is PPT].



(III) Lipids

Lipids constitute about 25% of diet, consist of triacylglycerols, cholesterol, and phospholipids.

1) TRIACYLGLYCEROLS:

- **Oral cavity:**

There is lingual lipase, but not important in humans.

- **Stomach:**

Gastric lipase (pH 3-6) has mild lipolytic action on primary ester link, [3]. It inhibited by gastric HCl, so its time action is limited especially in adults.

So, some free acids and 1, 2 diacylglycerols are released.

- **Small intestine:**

Duodenum:

Strong pancreatic lipase (pH8) is activated by bile salts, Ca^{++}, and colipase (protein in pancreatic secretion).

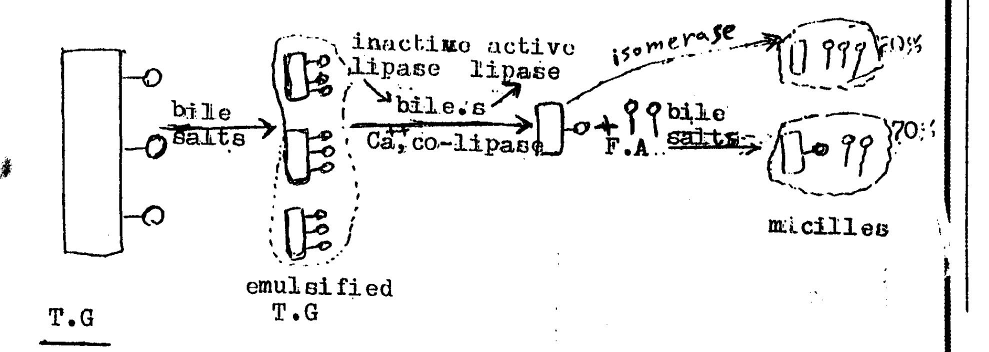
Inactive pancreatic lipase $\xrightarrow[\text{CO lipase}]{\text{bile salts } Ca^{++}}$ active lipase acts on primary links (3,1) to yield → free fatty acids, 2 monoacyl glycerols.



Secondary ester link (2) is difficult to separates, so it changed into 1.monoacylglycerol by isomerase which is early attacked by pancreatic lipase.

2.monoacylglycerol $\xrightarrow{\text{isomerase}}$ 1.Monoacylglycerol $\xrightarrow[\text{lipase}]{\text{panc.}}$ F.F A+ glycerol

About 70% of lipids are changed into 2.monoacylglycerol, but only 20% are completed to glycerol and F.F. acids.



EMULSIFICATION:

Lipids are first emulsified with bile salts, in which the large fat molecule is broken-down into small ones to ↑ its surface area to act upon by lipase.

Micelles:

When lipids are digested by pancreatic lipase, it will be surrounded by bile salts to be more water soluble for easily absorption (by ↓ its surface tension).

So, the end products are: F.F.A, 2.monoacylglycerol (70%), and glycerol (20%)

2) PHOSPHOLIPIDS:

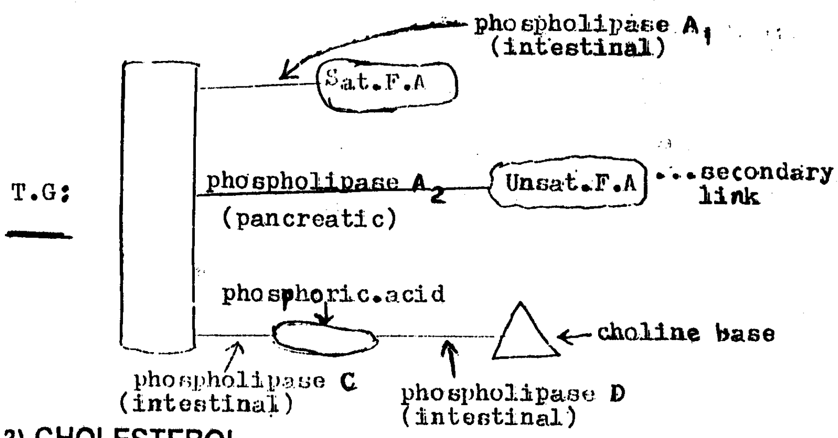
Are conjugated lipids formed of:

1- glycerol, 2 fatty acids (sat., unsat.)

2- phosphoric acid

3- base (choline or colamine)

Phospholipids are digested by pancreatic and intestinal phospholipases (A_1, A_2, C, D) in intestine into: glycerol, sat. F. acid, unsat. F. acids, phosphoric acid and the base.



3) CHOLESTEROL:

Free cholesterol is absorbed as such, but cholesterol ester undergoes hydrolysis by pancreatic cholesterol hydrolase (esterase) yielding free cholesterol and free fatty acid.

The end products of all lipid digestion are lonanged into micelles by aid of bile salts to be water soluble for its absorption in jejunum and ileum.



ABSORPTION

- In mucosal cells, long chain fatty acids are activated by [thiokinase, ATP, CoA.SH) and combine with 2, monoacylglycerol forming triacylglycerol molecule again (resynthesis), through acyl transferase.
- Phospholipids are also resynthetized in the mucosal cells.
- The resynthetized triacylglycerol, phospholipids, and cholesterol are bound to a mucosal protein (Apolipoprotein B_{48}) to form large lipoprotein molecule (chylomicrons) which enter circulation via lacteals and the thoracic duct.
- Chylomicrons may cause plasma turbidity if increased (has high content of triacylglycerol)
- The apolipoprotein B surrounds fats from outside to be hydrophilic in blood (water soluble)
- In the blood other layers of apolipoproteins are added according to type of lipoproteins.
- Therefore, lipids in the blood are present in different forms of lipoproteins (chylomicrones, β, pre β and alpha)

- <u>Steatorrhe</u>:

Is condition in which fat content in stool is increased above 5-6gm/day, due to lack of bile salts or pancreatic lipase or due to unhealthy endothelium (sprue).





(IV) Nucleoproteins

Are conjugated proteins, formed of basic proteins (rich in basic amino acids) and nucleic acid.

Nucleoproteins are present in all mammalian and plant cells, viruses and bacterial.

They take part in cell division, carry the genes in nuclei, or take part in protein biosynthesis in cytoplasm (RNA).

The protein part of nucleoproteins is digested in stomach and intestine as any protein.

The nucleic acid:

The nucleic acid in duodenum is attacked by pancreatic nucleases [ribonuclease for RNA, deoxynuclease for DNA) producing the polynucleotides.

Nucleotides:

Then, the intestinal phosphatase (pH 8.6) acts on nucleotides yielding nucleoside, and free phosphate.



Nucleosides:

- The intestinal nucleosidases act on nucleosides yielding pentose sugar, and free bases [purine or pyrimidine].
- Therefore, the end result of digestion is purine and pyrimidine bases, pentosis, and phosphates.





SUMMARY OF DIGESTION:

- *Stomach*: Digestion of protein part of nucleoproteins → basic amino acids.
- *Duodenum*: pancreatic ribo and deoxynuclease on nucleic acid → polynucleotides.
- *Jejunum & ileum*: 1) Polynucleotidase: on polynucleotides → mononucleotides.
  2) Phosphatase: on mononucleotide → nucleoside + phosphate
  3) Nucleosidase: on nucleoside → pentose (ribose & deoxyribose) and base (purine & pyrimidine)



ABSORPTION OF NUTRIENTS:

Jejunum:

- Glucose & other monosaccharides.
- Fatty acids & cholesterol.
- Amino acids & short peptides.
- Vitamins & folate
- Electrolytes, iron, Ca^{++}, Water

Ileum:

- Bile acids (retrohepatic circulation)
- Vit. B_{12}
- Electrolytes and water



رقم الإيداع بدار الكتب : 04/22148

Biological Membranes: Structure and Function

Plasma membrane forms enclosed compartment around each cell and forms a barrier between inside and outside of the cell. Membranes also form specialized compartments within the cells e.g. nucleus, mitochondria, lysosomes....

Membranes are composed of lipids, proteins and carbohydrates. The relative content of these components varies widely from one type of membrane to another, but typically it contains 40% of the dry weight is lipids, about 60% proteins and 1 to 10% carbohydrates. All membrane carbohydrate is covalently attached to proteins or lipids.

COMPOSITION OF DIFFERENT MEMBRANES: CONTENT OF LIPID, PROTEIN AND CARBOHYDRATES AS PERCENTAGE OF DRY WEIGHT

Type of membranes	*Lipid*	*Protein*	*Carbohydrate*
• Plasma membrane (mammals)	43	49	8
• Nuclear membrane	35	59	3
• Outer mitochondrial membrane	48	52	Trace
• Inner mitochondrial membrane	24	76	Trace
• Endoplasmic reticulum	44	54	2
• Myelin	75	22	3

I- Membrane Lipids

Lipid Bilayer of Membranes

The major lipids in mammalian membranes are phospholipids, glycolipids and cholesterol. In aqueous solutions, amphipathic molecules are oriented in such a way to prevent the hydrophobic region from coming into contact with water. The amphipathic characters of phospholipids make them to form a lipid bilayer in aqueous medium. Once formed, the bilayer structure is maintained by multiple noncovalent interactions, which include the followings:

- Hydrophobic interactions and Van der Waal's forces between the hydrocarbon chains of fatty acids.
- Ionic interactions and hydrogen bonding between the polar groups.
- Hydrogen bonding between polar groups and water.

Saturated fatty acids (SFA) have straight tails, whereas cis-unsaturated fatty acids (USFA) have kinked tails. As more kinks are present in the membrane it becomes less tightly packed and therefore more fluid.

A- Phospholipids

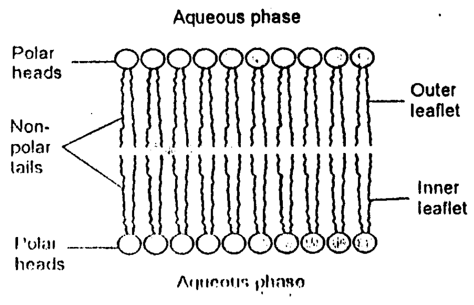
The main types of phospholipids are phosphoglycerides containing ethanolamine, choline, serine or inositol. Choline containing phospholipids (phosphatidyl choline and sphingomyelin) are located mainly in the outer leaflet, while aminophospholipids (phosphatidyl serine and ethanolamine) are located mainly in the inner leaflet. Sphingomyelin is present specifically at high concentrations in myelin sheaths (hence its name). The fatty acids present are mainly C16 and C18. Phospholipids are mainly responsible for membrane fluidity (due to presence of USFA) and asymmetry (due to different distribution on both surfaces).

B- Glycolipids

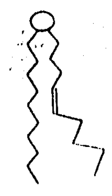
These are mainly in the form of cerebrosides and gangliosides.

C- Cholesterol

It is present mainly in plasma membrane, lesser amounts are found in mitochondrial and nuclear membranes. It is more abundant toward the outside of the plasma membrane. Its rigid sterol ring interacts with the acyl chains of phospholipids, limit their movement and decreases membrane fluidity at higher temperatures. At lower temperatures, it assists in keeping the membrane fluid and lowers the temperature at which the fluid to gel transition occurs.



PHOSPHO.LIPID BILAYER

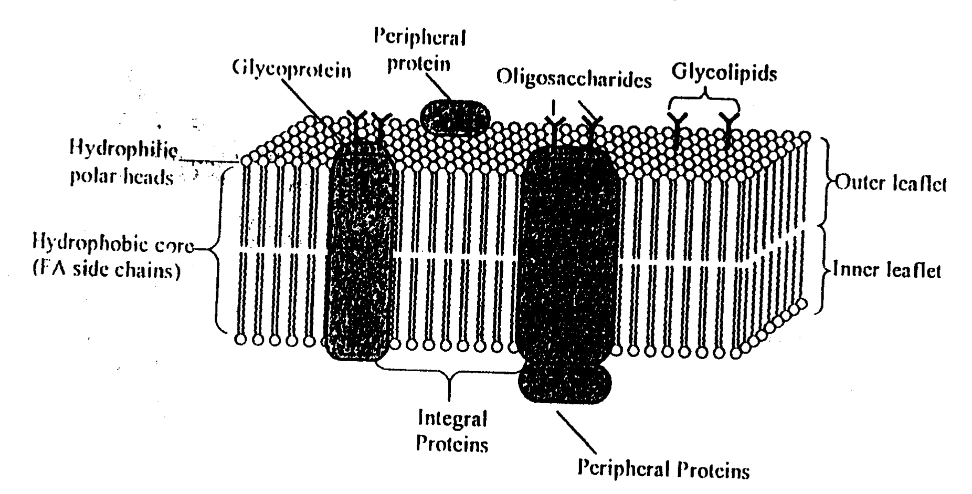


Hydrocarbon tail
SFA USFA

COMPOSITION OF DIFFERENT MEMBRANES: CONTENT OF VARIOUS LIPIDS AS PERCENTAGE OF TOTAL LIPIDS

Type of Membranes	*Various types of lipids*					
	Cholesterol	*Lecithin*	*Cephalin*	*Phosphatidyl serine*	*Sphingo myelin*	*Glycolipid*
• Plasma membrane (mammals)	20	19	12	7	12	10
• Nuclear membrane	3	45	20	3	2	0
• Outer mitochondrial membrane	8	45	20	2	4	0
• Inner mitochondrial membrane	0	35	25	0	3	0
• Endoplasmic reticulum	5	48	19	4	5	0
• Myelin	28	11	17	6	7	29

II- Membrane Proteins



The fluid mosaic model of membrane structure

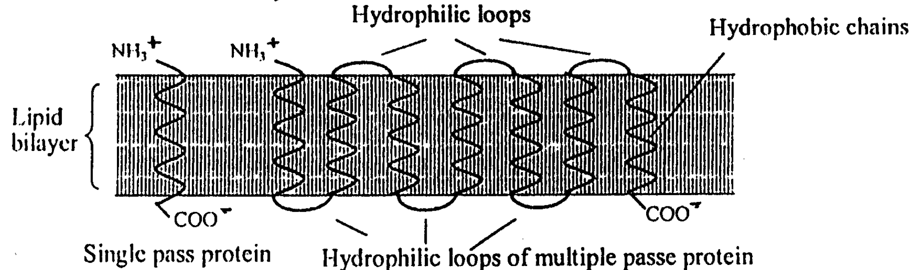
Membrane phospholipids act as a solvent for membrane proteins forming an environment in which proteins can function. Proteins are arranged in a way that their hydrophobic regions are traversing the hydrophobic core of the phospholipid bilayer **(Fluid mosaic model)**.

Types of Membrane Proteins

There are mainly two types, integral and peripheral.

A- Integral proteins (Transmembrane proteins)

These are usually globular and amphipathic. Most of them traverse the membrane and have two hydrophilic ends separated by hydrophobic region. They may have one **single pass or multiple passes** through the lipid bilayer. They require detergents for their release, which destroy the membrane.



B- Peripheral proteins

They are weakly bound to the hydrophilic region of an integral protein. They are released by treatment with high concentration of salts without destroying the membrane and do not require detergent.

Importance of Membrane Proteins

1- **Receptors:** many membrane hormonal receptors are integral proteins e.g. insulin and glucagon receptors.

2- **Transporters:** they are important for different membrane transport mechanisms e.g. glucose transporters and Na^+ - K^+ pump.

3- **Enzymes:** many of them form different enzymes of membrane e.g. ATP synthase present in the inner mitochondrial membrane.

4- **Tissue respiration:** mitochondrial membrane proteins form the different components of respiratory chain, which are important for oxygen utilization and energy generation.

5- **Structural elements** of many cells e.g. ankyrin, actin and spectrin which are the principal proteins forming the erythrocyte cytoskeleton.

6- **Immunoglobulin molecules** on the plasma membrane of lymphocytes are integral proteins and can be released by the shedding of small fragments of the membrane.

III- Membrane Carbohydrates

They are present as part of membrane glycolipids and glycoproteins. Because sugars are very hydrophilic, they are located at or projecting from the outer surface of the membrane.

Importance of membrane carbohydrates

1- They are important constituents of cell membrane glycoproteins, which are essential in cell recognition processes (receptors) and transport mechanisms.

2- They are important constituents of glycophorin present in red cell membrane.

3- They are critical in the interaction between sperms and ovulated eggs. The later contain glycoproteins that are recognized by receptors on the surfaces of sperms. Their binding triggers the release of sperm enzymes (hyaluronidase and proteases) that allow sperm entry.

Membrane Fluidity

The degree of fluidity of a biological membrane is a function of both temperature and lipid composition. It is largely determined by percentage of USFA in its phospholipid molecules. The hydrocarbon chains of USFA (due to presence of kinks of double bonds) pack less densely than SFA and increase membrane fluidity. Cholesterol rigid ring moderates membrane fluidity by interrupting the lateral diffusion of nearby phospholipids.

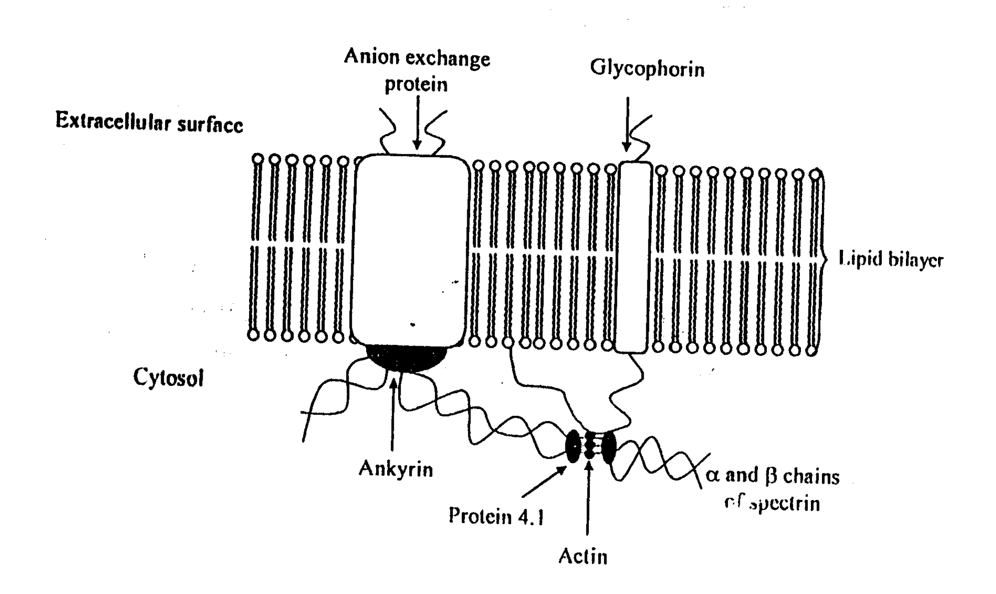
Diseases Due to Mutations Affecting Genes Encoding Membrane Proteins

Proteins in membranes are classified as receptors, transporters, ion channels, enzymes and structural elements. So gene mutations can affect these proteins and produce diseases. For example:

1- **Familial hypercholesterolemia**: It is due to mutation in the gene encoding LDL (low density lipoprotein) receptors, which are essential for uptake of this plasma lipoprotein by liver and extrahepatic cells and for removal of cholesterol from plasma, resulting in elevated plasma cholesterol level.

2- **Hereditary spherocytosis**: It is due to mutation in the gene encoding spectrin, which results in loss of the specific erythrocyte shape, reduction of red cell life span and anemia.

ERYTHROCYTE MEMBRANE



Red Cells Membranes

The same integral proteins and peripheral proteins, as discussed above, are present in cell membrane of nearly all vertebrate erythrocytes. The nature and function of these proteins require special mention;

I- Lipid Components

- The major lipids are phospholipids and cholesterol.
- The major phospholipids are phosphatidyl-choline, phosphatidyl-serine, phosphatidyl-ethanolamine and sphingomyelin.
- Choline containing phospholipids predominate in the outer leaflet (phosphatidyl-choline and sphingomyelin), while phosphatidyl-serine and phosphatidyl-ethanolamine predominate in the inner leaflet.
- Glycolipids including **the ABO blood groups** form about 5 - 10 % of the total lipid contents.

II – Protein Components

A- Integral Proteins

The major integral proteins include the following:

1- Anion exchange protein

It is a transmembrane glycoprotein. It forms a tunnel permitting the exchange of chloride for bicarbonate. It is a multipass membrane protein, extending across the bilayer at least ten times. Its cytosolic amino terminal binds many proteins; hemoglobin, ankyrin and several glycolytic enzymes.

2- Glycophorins

They are transmembrane proteins of a single pass type, and are heavily glycosylated (60 %). The carbohydrate groups of glycophorins are rich in sialic acid (a negative charged sugar), which give the red cells a very hydrophilic charged coat. This enables them to circulate without adhering to other cells or vessel walls.

3- Glucose transporters

Each is formed of about 12 transmembrane helical segments. It forms a gate that allows the passage of glucose. It is conformationally dependent on the presence of glucose (**facilitated diffusion**) and can oscillate rapidly (about 900 times / s).

B- Peripheral Proteins

They play an important role in maintenance of shape and flexibility of red cells. The cytosolic surface of the erythrocyte membrane is covered by a network of peripheral membrane proteins that make up the cytoskeletal structure (cytoskeleton). They mainly include the following:

1- Spectrin

It is the major protein of cytoskeleton. It is composed of two polypeptide chains:α and β chains. These chains are arranged in an antiparallel manner, they are interwined forming a dimer. They inter-act head to head to form a tetramer. At the tail, they bind to actin and protein 4.1. Also, it is bounded to ankyrin that connect it to the cell membrane.

2- Ankyrin

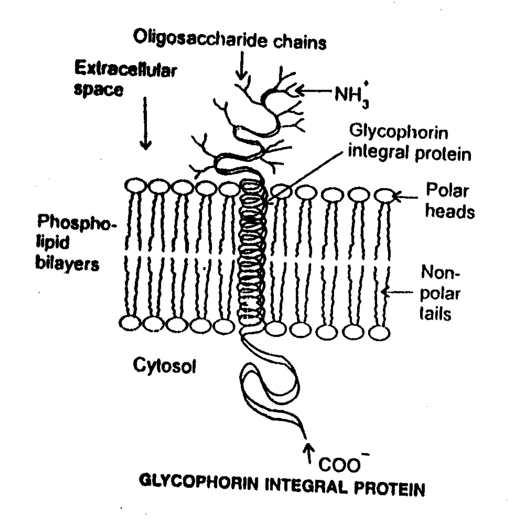
It is a protein that bound spectrin to cell membrane.

3- Actin

It exists as short double helical filaments (F-actin). It binds to the tail end of spectrin dimer and protein 4.1.

4- Protein 4.1

It is a globular protein. It binds tightly to the tail end of spectrin near the actin binding site to form protein 4.1-spectrin-actin complex. Protein 4.1 binds also to the integral membrane proteins and to the membrane phospholipids, thus connecting the cytoskeleton to the membrane proteins and lipid bilayer.



GLYCOPHORIN INTEGRAL PROTEIN

Clinical Aspect

Hereditary spherocytosis and hereditary elliptocytosis are inherited genetic abnormalities of red cells in which red cells are of abnormal shape. In hereditary spherocytosis the red cells are spherical and in hereditary elliptocytosis they are ellipsoidal. These defects in shape of red blood cells lead to increased haemolysis, anaemia and jaundice. These abnormally shaped red blood cells are fragile and have shorter life than normal erythrocytes.

Defect: They result from mutations in the genes coding for proteins of the membrane. The abnormality may be from defective spectrin that is unable to bind either ankyrin or band 4, 1 protein and in some cases band 4, 1 protein is absent.

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